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(54) Title: OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS HYPERSENSITIVE RESPONSE ELICITOR

(57) Abstract: The present invention relates to a chimeric gene that includes a first DNA molecul encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulat ry regi n operably linked to the first DNA molecule. Also disclosed are an expression system and a host cell containing the chimeric gene. The present invention also relates to a transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant including the chimeric gene, wherein the promoter induces transcription of the first DNA molecule in response t infection of the plant by an oomycete. Transgenic seeds and transgenic cultivars obtained from the transgenic plant are also disclosed. Additional aspects f the present invention include methods of making a recombinant plant cell and a transgenic plant.

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OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS HYPERSENSITIVE RESPONSE ELICITOR

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/178,565, filed January 26, 2000, which is hereby incorporated by reference in its entirety.

This invention was made in part with support by the U.S. Government under Grant No. 97-34367-3937 from the U.S. Department of Agriculture. The U.S. Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to transgenic plants resistant to oomycete infection which contain a heterologous hypersensitive response elicitor under the control of a promoter responsive to infection by an oomycete.

BACKGROUND OF THE INVENTION

In general, fungal plant diseases can be classified into two types: those caused by soilborne fungi and those caused by airborne fungi. Soilborne fungi cause some of the most widespread and serious plant diseases, such as root and stem rot caused by *Fusarium spp.* and root rot caused by *Phytophthora spp.* For example, *Phytophthora parasitica* var. *nicotiana,* a soilborne oomycete found in many tobacco growing regions worldwide, causes black shank, a highly destructive root and stem rot disease of many varieties of cultivated tobacco.

Since airborne fungi can be spread long distances by wind, they can cause devastating losses, particularly in crops which are grown over large regions. A number of pathogens have caused widespread epidemics in a variety of crops. Important diseases caused by airborne fungi are stem rust (*Puccinia graminis*) on wheat, corn smut (*Ustilago maydis*) on corn, and late blight disease (*Phytophthora infestans*) on potato and tomato. *Plasmopera viticola* is an airborne oomycete that causes downy mildew disease on grape vines. The blue mold fungus (*Peronospora*

tabacina) has caused catastrophic losses in tobacco crops, particularly in the United States and Cuba.

Most of these fungal diseases are difficult to combat. and farmers and growers must use a combination of practices, such as sanitary measures, resistant cultivars, and effective fungicide against such diseases. Hundreds of millions of dollars are spent annually for chemical control of plant-pathogenic fungi. As a result, there is today a real need for new, more effective and safe means to control plant-pathogenic fungi, particularly oomycetes which are responsible for major crop loss.

Genetic engineering promises to be an effective strategy for reducing the losses associated with diseases of field crops. Several successful approaches have been reported where the constitutive expression of antimicrobial peptides such as cecropins (Arce et al., "Enhanced Resistance to Bacterial Infection by Erwinia Carotovora Susp. Atroseptica in Transgenic Potato Plants Expressing the Attacin or the Cecropin SB-37 Genes," Am. J. Potato Res. 76:169-177 (1999)), lysozyme (Nakajima et al., "Fungal and Bacterial Disease Resistance in Transgenic Plants Expressing Human Lysozyme," Plant Cell Reports 16:674-679 (1997)), and monoclonal antibodies (Tavladoraki et al, "Transgenic Plants Expressing a Functional Single Chain FV Antibody are Specifically Protected from Virus Attack," Nature 366:468-472 (1993)) effectively protected plants from parasitic organisms. However successful, these approaches have limited application to food production since many of these antimicrobial peptides and plant defense molecules are potentially toxic or allergenic to humans (Franck-Oberaspach et al., "Consequences of Classical and Biotechnological Resistance Breeding for Food Toxicology and Allergenicity," Plant Breeding 116:1-17 (1997)). Thus, alternative approaches for genetically engineering disease resistance would be more desirable.

Plants posses a highly evolved pathogen surveillance system which allows for recognition of specific pathogen derived molecules known as elicitors. Elicitor recognition results in an incompatible plant-microbe interaction, defined as the rapid activation of plant defense genes, typically resulting in the hypersensitive response and the onset of systemic acquired resistance.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,

"Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al., "Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, (1982), these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants

and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); Wei. Z.-M., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M., et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of Pseudomonas solanacearum," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 *PopA*1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al., "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of Pseudomonas solanacearum," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91

(1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N$_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

Because the hypersensitive response results in localized necrosis of plant tissue, it is desirable to limit expression of a heterologous hypersensitive response elicitor to certain tissues in transgenic plants. This approach is discussed generally in PCT publication WO 94/01546 to Beer et al., but no specific transgenic plants are identified and only two suitable fungus-responsive promoters are suggested, e.g., the phenylalanine ammonia lyase and chalcone synthase promoters. No promoters responsive specifically to infection by oomycetes are identified therein.

The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to a chimeric gene that includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. Also disclosed are an expression system that includes a vector in which is inserted a chimeric gene of the present invention and a host cell that includes a chimeric gene of the present invention.

Another aspect of the present invention relates to a transgenic plant resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

Transgenic seeds and transgenic cultivars obtained from the transgenic plant are also disclosed.

An additional aspect of the present invention relates to a method of making a recombinant plant cell. This is accomplished by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.

A further aspect of the present invention relates to a method of making a plant resistant to disease resulting from oomycete infection. This is accomplished by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating the plant from the transformed plant cell.

The present invention confers oomycete-induced disease resistance to plants transformed with a chimeric gene encoding a hypersensitive response elicitor protein or polypeptide, which is transcribed within a limited population of plant cells in response to infection of the plant by an oomycete. To limit transcription of the chimeric gene within a certain population of plant cells, the chimeric gene includes a promoter that is responsive to infection by an oomycete (i.e., it is activated by the oomycete). The hypersensitive response elicitor protein or polypeptide can cause tissue collapse at the site of infection and/or induce systemic resistance against the oomycete and other pathogens. By using the promoter from the potato *gst1* gene, for example, which is activated by infection with oomyceteous fungi, the present invention can control fungal pathogens within crops without harming the transgenic plant and without resorting to use of environmentally damaging chemicals.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation and partial restriction map of T-DNA in plant transformation vector pCPP1294. Filled triangles represent the left and right borders; *Pgst1* represents the *gst1* promoter from potato variety Atlantic; PR1-b represents the DNA molecule encoding a signal sequence from *Nicotiana tabacum*;

hrpN represents the DNA molecule encoding the hypersensitive response elicitor harpin$_{Ea}$ of *Erwinia amylovora*; NT represents the nos terminating region; *aacC1* represents the gentamycin resistance cassette.

Figure 2 is an image of transgenic *Arabidopsis* plants containing a construct encoding GUS under control of the *gst1* promoter. To demonstrate pathogen inducibility of the *gst1* promoter in *Arabidopsis*, GUS staining was measured following inoculation of the plants with water (left) or *P. parasitica* (right). GUS expression is indicated by dark staining.

Figures 3A and 3B show an analysis of *hrpN* gene expression in *Arabidopsis* transgenic line GSSN8-4, containing the construct shown in Figure 1, after inoculation with *P. parasitica* NOCO. At one day intervals leaves were collected for isolation of total RNA. Figure 3A is a Northern blot analysis performed using *hrpN* DNA as a probe. Figure 3B is an ethidium bromide stained gel shown as a control (bottom).

Figures 4A and 4B are images demonstrating *Arabidopsis* GSSN 8-4 are resistant to *P. parasitica*. Figure 4A shows the effects of *P. parasitica* infection in WT Arabidopsis (control, left) and GSSN 8-4 Arabidopsis (test, right). Figure 4B shows the degree of trypan blue staining of *P. parasitica*-infected leaves of WT (control, left) and GSSN 8-4 plants (test, right), both taken 10 days post-inoculation.

Figure 5 is a graph depicting the severity of *P. parasitica* infection in WT (control), EV (control), and *hrpN* transgenic plants (test). Two week old plants were drop inoculated with conidiospores of *P. parasitica* (2 ml drops; 5 x 10^4 spores/ml). Ten days after inoculation, 30 plants of each genotype were rated for disease severity. Ratings were adapted from Cao et al. ("Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," Proc. Natl. Acad. Sci. USA 95:6531-6536 (1998), which is hereby incorporated by reference) as follows: 1, no conidiophores present on plant; 2, 0-5 conidiophores per infected plant; 3, 6-20 conidiophores present on a few infected leaves; 4, 6-20 conidiophores present on most infected leaves; 5, more than 20 conidiophores on all infected leaves.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention relates to a novel DNA construct in the form of a chimeric gene. The chimeric gene includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. As discussed more fully hereinafter, a chimeric gene of the present invention is particularly useful in preparing a transgenic plant for the purpose of rendering the transgenic plant resistant to disease resulting from infection thereof by an oomycete.

The first DNA molecule can encode any hypersensitive response elicitor protein or polypeptide which is effective in triggering a hypersensitive response (i.e., in a particular host plant selected for transformation). Generally, it is desirable to express hypersensitive response elicitors only in plants which are non-hosts for the source organism of the hypersensitive response elicitor. Suitable hypersensitive elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors from Gram-positive bacteria. One example is the hypersensitive response elicitor from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitins) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide of *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

The hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240
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Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

```
cgattttacc cgggtgaacg tgctatgacc gacagcatca cggtattcga caccgttacg   60
gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgtc  120
gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg  180
cagcaatatc ccggcatgtt gcgcacgctg ctcgctcgtc gttatcagca ggcggcagag  240
tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg  300
ccgtcggatc ccggcagtta tccgcaggtg atcgaacgtt tgtttgaact ggcgggaatg  360
acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc  420
cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacggtga ggaaccgttt  480
caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg  540
ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca  600
aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccggtctgg ggctgggtgc  660
tcagggactg aaaggactga attccgcggc ttcatcgctg ggttccagcg tggataaact  720
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct  780
ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc  840
tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga  900
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tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960
caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020
ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080
caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140
gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200
ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260
ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga 1320
tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440
cgccagcatg gacaaattcc gtcaggcgat gggtatgatc aaaagcgcgg tggcgggtga 1500
taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560
ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccctttag 1920
cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctgggcga cctgatcggt t 2141

The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
20 25 30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
50 55 60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
180 185 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
210 215 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
245 250 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
275 280 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
290 295 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305 310 315 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
325 330 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365

```
Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                 375                 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                 390                 395                     400

Gly Ala Ala
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa     60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct    120
atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg    180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg    240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggtgg tgggctgatg    300
ggcggtggct taggcggtgg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa    360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa    420
ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggtattaac    480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac    540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg    600
caagatggca cccagggcag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac    660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag    720
ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggtcttgac    780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag    840
ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat    900
atcggtacgc acaggcacag ttcaacccgt tctttcgtca ataaaggcga tcgggcgatg    960
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac   1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc   1080
```

aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140
atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260
cttggcaagc tgggcgcggc ttaagctt 1288

The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                   10                  15
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190
Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205
Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220
```

```
Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccc cggcaatggc ccttgtcctg    60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc   120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga   180
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc   240
atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg   300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc   360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac   420
gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc   480
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac   540
```

gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag 600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg 840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020
gcctga 1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference.

The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145 150 155 160

```
Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175
Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190
Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205
Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220
Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240
Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255
Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270
Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285
Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300
Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320
Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335
Gln Ser Thr Ser Thr Gln Pro Met
            340
```

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

```
atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc      60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc     120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc     180
ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc     240
```

```
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc   300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa   360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag   420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc   480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc   540
ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt   600
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac   660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac   720
cagggcggcc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag   780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc   840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat   900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc   960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg  1020
acgcagccga tgtaa                                                    1035
```

Other embodiments of the present invention include, but are not limited to, use of the nucleotide sequence encoding for the hypersensitive response elicitor protein or polypeptide from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

The hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi

Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet. et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants." Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

Other elicitors can be readily identified by isolating putative hypersensitive response elicitors and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art. The isolated DNA molecule can then be introduced into the chimeric gene for expression in a transgenic plant of the present invention.

The first DNA molecule can also encode fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding

editions), which are hereby incorporated by reference. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

The first DNA molecule also can be a DNA molecule that hybridizes under stringent conditions to the DNA molecule having nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, or 8. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the

temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 μg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

Variants of suitable hypersensitive response elicitor proteins or polypeptides can also be expressed by the first DNA molecule. Variants may be made by, for example, the deletion, addition, or alteration of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide (such as a 6xHis tag).

The promoter of the chimeric gene should be selected on the basis of its ability to induce transcription of the first DNA molecule in response to infection of the plant by an oomycete (i.e., the oomycete activates the promoter).

According to one embodiment, the promoter preferably includes some or all of the promoter-effective regions of a *gst1* gene from potato. The *gst1* promoter is activated in response to infection by oomycetes and not by wounding or other environmental perturbations. The *gst1* promoter from potato has a nucleic acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
gaattcagga agaattttgt aggttcaact aaattatata tatatatata aaaaaataaa    60
aattattaga cgcttcgact atttacttac tttaaaattt gaattttcgt acgaataaaa   120
ttatttgtca gagaaaagtc ttttagctat tcacatgcta ggaagtttca cttttggtgg   180
```

```
atcagtgatt gtatattatt taatatatat caattttctc atcaaactga aaatgaaaga    240
taaaattaat attaaaaact ccattcattt taatttattg tcatgttttg acttgatcca    300
aaatctaaca atttaaaagg ttttaaattt ttgtgctttt ttttaaatta aaaatatgtc    360
aaatatatta aaatatattt tttaaatttt atactaaaaa acatgtcaca tgaatatttg    420
aaattataaa attatcaaaa ataaaaaaag aatatttctt taacaaatta aaattgaaaa    480
tatgataaat aaattaaact attctatcat tgatttttct agccaccaga tttgaccaaa    540
cagtgggtga catgagcaca taagtcatct ttattgtatt ttattactca ctccaaaaat    600
atagggaata tgtttactac ttaatttagt caaatataat tttatattag aataattgaa    660
tagtcaaaca agaaacttta atgcatcctt attttt                              696
```

Effective fragments of SEQ. ID. No. 9 are also encompassed by the present invention. U.S. Patent Nos. 5,750.874 and 5,723.760 to Strittmayer et al.. which are hereby incorporated by reference, define promoter-effective regions of the potato *gst1* promoter. Preferably, the *gst1* promoter includes a nucleotide sequence corresponding, at a minimum, to nucleotides 295-567 of SEQ. ID. No. 9. The *gst1* promoter can also include effective portions containing nucleotides 295-696 of SEQ. ID. No. 9.

The chimeric gene of the present invention also includes an operable 3' regulatory region, selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA for expression in plant cells, operably linked to the first DNA molecule which encodes for a hypersensitive response elicitor. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l Acad. Sci. USA, 80:4803-4807 (1983). which is hereby incorporated by reference) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference). Virtually any 3' regulatory region known to be operable in plants would suffice for proper expression of the coding sequence of the chimeric gene of the present invention.

The first DNA molecule, promoter, and a 3' regulatory region can be ligated together using well known molecular cloning techniques described in

Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference.

The chimeric gene can also include a second DNA molecule encoding a secretion signal. A number of suitable secretion signals are known in the art and other are continually being identified. The secretion signal can be an RNA leader which directs secretion of the subsequently transcribed protein or polypeptide, or the secretion signal can be an amino terminal peptide sequence that is recognized by a host plant secretory pathway. The second DNA molecule can be ligated between the promoter and the first DNA molecule, using known molecular cloning techniques as indicated above.

According to one embodiment, the second DNA molecule encodes a secretion signal derived from *Nicotiana tabacum*. Specifically, this DNA molecule encodes the secretion signal polypeptide for *PR1-b* gene of *Nicotiana tabacum*. This second DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tctagaccat gggatttttt ctcttttcac aaatgccctc atttttcttt gtgtcgacac   60
ttctcttatt cctaataata tctcactctt ctcatgccca aaactctaga            110
```

The above sequence includes XbaI sites (underlined) at each end to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. The coding sequence of SEQ. ID. No. 10 starts at base 8. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser
 1               5                  10                  15

Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn
            20                  25                  30

Ser Arg
```

An alternative second DNA molecule encoding the secretion signal polypeptide for *PR1-b* gene of *Nicotiana tabacum* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

atgggatttt ttctcttttc acaaatgccc tcattttttc ttgtctctac acttctccta 60
ttcctaataa tatctcactc ttctcatgcc caaaactctc aa 102

This nucleotide sequence is disclosed in Genbank Accession No. X03465, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser
1 5 10 15

Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn
20 25 30

Ser Gln

Yet another second DNA molecule encodes the secretion signal for the *PR1-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 14 as follows:

atgggatttg ttctcttttc acaattgcct tcatttcttc ttgtctctac acttctctta 60
ttcctagtaa tatcccactc ttgccgtgcc 90

This DNA molecule is disclosed in Genbank Accession No. X06361, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu Leu Val Ser
1 5 10 15

Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg Ala
20 25 30

Still another second DNA molecule encodes the secretion signal for the *PR4-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 16 as follows:

atggagagag ttaataatta taagttgtgc gtggcattgt tgatcatcag catggtgatg 60
gcaatggcgg cggca 75

This DNA molecule is disclosed in Genbank Accession No. X58546, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

```
Met Glu Arg Val Asn Asn Tyr Lys Leu Cys Val Ala Leu Leu Ile Ile
 1               5                  10                      15

Ser Met Val Met Ala Met Ala Ala Ala
            20                  25
```

Each second DNA molecule can be cloned using primers that introduce restriction sites at the 5' and 3' ends thereof to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. SEQ. ID. No. 10 is shown to include such restriction sites (e.g., XbaI).

Further aspects of the present invention include an expression system that includes a vector containing a chimeric gene of the present invention, as well as a host cell which includes a chimeric gene of the present invention. As described more fully hereinafter, the recombinant host cell can be either a bacterial cell (i.e., *Agrobacterium*) or a plant cell. In the case of recombinant plant cells, it is preferable that the chimeric gene is stably inserted into the genome of the recombinant plant cell.

The chimeric gene can be incorporated into cells using conventional recombinant DNA technology. Generally, this involves inserting the chimeric gene into an expression vector or system to which it is heterologous (i.e., not normally present). As described above, the chimeric gene contains the necessary elements for the transcription and translation in plant cells of the first DNA molecule (i.e., encoding the hypersensitive response elicitor protein or polypeptide) and, if present, the second DNA molecule.

U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Once the chimeric gene of the present invention has been prepared, it is ready to be incorporated into a host cell. Recombinant molecules can be introduced

into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like. Preferably the host cells are either a bacterial cell or a plant cell.

Accordingly, another aspect of the present invention relates to a method of making a recombinant plant cell. Basically, this method is carried out by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter. Preferably, the chimeric gene is stably inserted into the genome of the recombinant plant cell as a result of the transformation.

A related aspect of the present invention concerns a method of making a plant resistant to disease resulting from oomycete infection. Basically, this method is carried out by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating a plant from the transformed plant cell.

One approach to transforming plant cells with a chimeric gene of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector

and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the chimeric gene is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the chimeric gene. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The chimeric gene may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the chimeric gene. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the chimeric gene into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the chimeric gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a chimeric gene of the present invention can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into

the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the chimeric gene of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that is resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete. Preferably, the chimeric gene is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce,

endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the chimeric gene is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

Resistance against different types of oomycetes may be imparted to transgenic plants according to the present invention. Without being bound by any particular theory, it is believed that a hypersensitive response elicitor protein or polypeptide encoded by the first DNA molecule is transcribed in response to infection of the plant by an oomycete. The exact mechanism by which the promoter is activated to regulate transcription of sequences under its control is not fully understood; however, the first DNA molecule is transcribed and the hypersensitive response elicitor is expressed in a limited population of cells (i.e., those in which transcription has been induced following oomycete infection). Once expressed, it is believed that the hypersensitive response elicitor can either be secreted from the plant cell (assuming the chimeric gene also contains a second DNA molecule encoding an N-terminal secretion signal) or leaked from an oomycete-infected plant cell.

Regardless of how the hypersensitive response elicitor is delivered to the intercellular environment, it is believed that the hypersensitive response elicitor protein or polypeptide will initiate a hypersensitive response to cause localized necrosis of oomycete-infected tissues. In addition, systemic acquired resistance may be developed by the transgenic plant following initiation of the hypersensitive response. This may yield broad disease and/or pathogen resistance to the transgenic plants of the present invention.

Oomycetes against which resistance is imparted include, without limitation, species of *Plasmopara, Phytophthora, Peronospora, Pseudoperonospora, Bremia, Sclerospora, Aphanomyces, Pythium*, and *Albugo*.

According to one embodiment of the present invention, an oomycete resistant transgenic tobacco plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of tobacco, including, but not limited to, *Peronospora tabacina* (which causes blue mold) and *Phyophthora parasitica* (which causes black shank).

The chimeric gene of the present invention can be utilized to impart oomycete resistance for a wide variety of tobacco plants, some of which may possess varying levels of natural resistance against pathogenic oomycetes. The varieties of tobacco plants which can be protected include, without limitation, those referred to as Coker 371 Gold, K 149, K 326, K 346, K 394, K 730, RG 11, RG17, RG22, Speight G-70, Speight G-117, Speight G-126, GL939, NC 55, NC 71, NC 72, NC 95, NC 2326, OX 207, OX 940, RG 81, RG H4, RG H61, Speight 168, Speight NF3, Speight 172, CU 236, CU 387, CU 368, NC TG91, OX 4142NF, OX 4083, RG 4H2-12, RG 4H2-17, RG 4H2-20, Speight 177, Speight 178, Speight 179, VPI 107, VPI 605, NG TG94, KY 14, KY 8959, KY 907, KY 908, TN 86, TN 90, TN 97, VA 116, VA 509, B 21 x KY 10, KY 14 x L8, NC 3, NC BH129, DH332, COOP 313, COOP 543, Clay's 403, Clay's 502, HY 402, PF 561, and R 711.

According to another embodiment of the present invention, an oomycete resistant transgenic grape plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of grape,

including, but not limited to, *Plasmopara viticola* (which causes downy mildew), *Pythium* spp. (which cause root and/or stem rot), and *Phytophthora* spp. (which cause root and/or stem rot).

The chimeric gene of the present invention can be utilized to impart oomycete resistance for a wide variety of grapevine plants. The chimeric gene is particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include, without limitation, those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Cornish, Black Damascus. Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight. Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include, without limitation, those used in wine production, such as Aleatico, Alicante Bouschet. Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat,

Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis california*, and *Vitis girdiana*.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., scion or rootstock cultivars) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart oomycete resistance to plants.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention, but they are by no means intended to limit its scope.

Example 1 - Construction of Chimeric Gene

Cloning of *gst1* promoter

The *gst1* promoter region from nucleotides (539 to +48) (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236 (2-3):179-86 (1993), which is hereby incorporated by reference), was PCR amplified using DNA from potato cultivar Atlantic, using a forward primer containing a BamHI site (SEQ. ID. No. 18) as follows:

tgacggatcc taggaagttt cacttttggt gg 32

a reverse primer containing an EcoRI site (SEQ. ID. No. 19) as follows:

tagcgaattc tatgtgtggt tggtctcccct tg 32

and PrimeZyme DNA polymerase (Whatman Biometra, Goettingen, Germany). The DNA was ligated into the LITMUS 38 vector (New England Biolabs, Beverly, MA) and three clones were sequenced on an ABI 377 sequencer at the Cornell BioResource Center. Each clone had two to three nucleotide changes when compared to the published sequence (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). The changes were most likely due to mistakes made by the polymerase because the promoter is extremely A-T rich and all but one of the changes were in different places in the three clones. One clone, pCPP1308, with a single change in the cis-acting region identified by Martini et al. ("Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference) was used as the source of the *gst1* promoter in all subsequent constructions.

Plant Transformation Constructs

The *gst1:uidA* construct was made by ligating the *gst1* promoter from pCPP1308 into pBI101 (Clontech Labs, Palo Alto, CA). For the *gst1:hrpN* and *gst1*:signal sequence:*hrpN* constructs (described below), the *gst1* promoter region was engineered to have a 5' HindIII site and a 3' XbaI site by the polymerase chain reaction (PCR) using pCPP1308 as the template. The forward primer had the nucleotide sequence of SEQ. ID. No. 18 and the reverse primer had a nucleotide sequence according to SEQ. ID. No. 20 as follows:

32

For *gst1:hrpN* constructs. the *hrpN* gene of *Erwinia amylovora* (i.e., encoding a hypersensitive response elicitor identified as $harpin_{Ea}$) was engineered to have a 5' XbaI restriction site and a 3' SstI restriction site by PCR using pCPP1084 (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia Amylovora," Science 257:85-88 (1992), which is hereby incorporated by reference) as the template. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 21 as follows:

```
atactctaga accatgggtc tgaatacaag tggg                                  34
```

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

```
tcatgagctc ttaagccggc ccagcttgcc aagtg                                 35
```

For *gst1*:signal sequence:*hrpN*, the *hrpN* gene was engineered to have a BamHI site on each end. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 23 as follows:

```
tagaggatcc ctgaatacaa gtgggctggg agcg                                  34
```

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

```
tcatggatcc ttaagccgcg cccagcttgc caagtg                                36
```

The nopaline synthase terminator was extracted from pBI101 by digesting with SstI and EcoRI.

The nucleic acid molecule encoding the PR1-b signal sequence (of SEQ. ID. No. 11) was engineered to have XbaI restriction sites on both ends. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 25 as follows:

atactctaga ccatgggatt ttttctcttt tca 33

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 26 as follows:

aggtctagag ttttgggcat gagaagagtg 30

The fragment was amplified using pSKG55 as a template (Gopalan et al., "Expression of the Pseudomonas Syringae Avirulence Protein AvrB in Plant Cells Alleviates its Dependence on the Hypersensitive-Response-and-Pathogenicity (Hrp) Secretion System in Elicitating Genotype-Specific Hypersensitive Cell Death." Plant Cell 8:1095-1105 (1996), which is hereby incorporated by reference).

PrimeZyme DNA polymerase (Whatman Biometra, Goettingen, Germany) was used with a hot start procedure for amplification of all fragments. The amplified fragments were purified, digested with the appropriate enzymes, and ligated into the binary vector pPZP221 (Hajdukiewicz et al., "The Small Versatile pPZP Family of Agrobacterium Binary Vectors for Plant Transformation," Plant Mol. Bio. 25:989-994 (1994), which is hereby incorporated by reference) or intermediate constructs, to build up the final constructs. The proper construction of pCPP1294 (Figure 1) was confirmed by sequencing on an ABI 377 automated sequencer.

The final constructs were transformed into Agrobacterium tumefaciens strain GV3101 (Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby incorporated by reference) by electroporation using a Bio-Rad GenePulser (Bio-Rad Ltd., York, UK).

Example 2 - Inoculation with *Peronospora parasitica* Activates gst1 Transcription in *Arabidopsis*

To evaluate the activity of the *gst1* promoter in a plant other than potato, transgenic Arabidopsis were constructed containing the *E. coli uidA* gene for ß-glucuronidase (GUS) under control of the *gst1* promoter. Histochemical GUS

assays of were performed essentially as described by Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby incorporated by reference. Uninoculated and inoculated whole small *Arabidopsis* plants were submerged for 30 minutes on ice in six well microtiter plates in a solution of 1.5% freshly prepared paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.2, containing 0.1% Triton X-100. The plants were washed twice for 5 minutes with sodium phosphate buffer pH 7.2. The plants were then submerged in a solution of 2 mM X-gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide), 50 mM sodium phosphate, pH 7.2, 0.5% Triton X-100. The solution was vacuum infiltrated into the plants and the plants were then incubated for 16 hours in the dark at 37°C. The staining was stopped by rinsing the plants several times in water and the tissue was then cleared by incubating in several changes of 70% ethanol.

Twenty lines were evaluated for GUS expression in uninoculated leaves, leaves inoculated with *Peronospora parasitica* isolate NOCO, and whole plants using a histochemical staining procedure (Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp 23-43 (1992), which is hereby incorporated by reference). Five lines showed more intense staining of the inoculated areas than the uninoculated areas and two lines showed no visible staining of any plant parts except the inoculated leaves (Figure 2). These results are consistent with those reported for potato and reveal that the gst1 promoter is pathogen inducible in *Arabidopsis*. No induction of GUS activity was detected in the five lines that responded to *P. parasitica* when inoculated with *Pseudomonas syringae* pv. tomato strain DC3000, even after disease symptoms appeared (results not shown). Previously, it was reported that the gst1 gene is induced in response to fungi, viruses, and nematodes (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato gst1 Gene," Mol. Plant-Microbe Interact. 9:68-73 (1996), which is hereby incorporated by reference), but results with bacterial pathogens were not reported.

Example 3 - **Pathogen Inducible Expression f *hrpN* in Transgenic *Arabidopsis***

To generate transgenic Arabidopsis expressing *hrpN* in a pathogen-inducible manner, plant transformation vectors, pCPP1292 for cytoplasmic localization of HrpN in plants, and pCPP1294 for extracellular localization of HrpN. were constructed. (Figures 3A and 3B). *Arabidopsis* ecotype Columbia (Col-0) was transformed with the two constructs. *Arabidopsis thaliana* ecotype Columbia (Col-0) plants were grown in a growth chamber at 22° C and a 17 hour photoperiod. Plants with primary fluorescence 5-15 cm tall were transformed via a known vacuum infiltration method (protocol available on the Internet at http://www.bch.msu.edu/pamgreen/vac.htm, which is hereby incorporated by reference) adapted from Bechtold et al., C. R. Acad. Sci. Paris 316:1194-1199 (1993). and Bent et al., Science 265:1856-1860 (1994), which are hereby incorporated by reference. Seeds were collected from each plant individually, sterilized and spread on selection plates containing 150 mg/l gentamycin, 0.2 g/l Arabidopsis Growth Medium (Lehle Seeds), and 0.7% Phytagar (Gibco BRL, Bethesda, MD). Plates were vernalized for 2 days at 4°C and then moved to a growth chamber maintained at 22° C and 14 hours light. Gentamycin resistant plants were selected after 2 weeks and individual plants were transplanted to soil. Each individual T1 seedling was brought up by single seed descent and individual plant lines were selected for lack of segregation of gentamycin resistance in the T3 generation. Insertion of T-DNA was confirmed by PCR and Southern analysis.

Transgenic *Arabidopsis* lines were inoculated 2 weeks after sowing with a 5 x 10^4 conidiospore suspension of *P. parasitica* isolate NOCO. Flats were covered with a humidity dome and moved to the growth chamber maintained at 18° C, 16 hours light, and 100% humidity. Plants were scored for infection 7 days after inoculation with a disease rating system adapted from Cao et al., "Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," Proc. Natl. Acad. Sci. USA 95:6531-6536 (1998), which is hereby incorporated by reference. A rating of 1, 0 conidiophores present; 2, 0-5 conidiophores present; 3, 6-20 conidiophores on a few leaves; 4, 6-20 conidiophores on all leaves; 5, 20 or more conidiophores present on all leaves. Inoculated leaves were stained with lactophenol-trypan blue (Keogh et al.,

"Comparison of Histological and Physiological Responses to Phakopsora Pachyrhizi in Resistant and Susceptible Soybean," Trans. Br. Mycol. Soc. 74:329-333 (1980), which is hereby incorporated by reference) to observe the extent of fungal colonization under the microscope.

Plants were selected that lacked segregation of antibiotic resistance in the T3 generation. Lines containing the *gst1:hrpN* construct ("GN lines") lines were tested for resistance to *P. parasitica* isolate NOCO in an initial screen.

Thirty lines containing the *gst1*:signal sequence:*hrpN* construct ("GSSN lines") were tested for resistance to *P. parasitica* isolate NOCO in an initial screen. All but one of the lines was free of any signs of the oomycete ten days after inoculation. Ten GSSN lines were chosen for further study and inoculated by spraying with a conidiospore suspension (5×10^4 spores/ml) of *P. parasitica* NOCO. Northern analysis revealed that expression of *hrpN* was induced by *P. parasitica* 2 days after inoculation with strong induction at 4 days (Figure 3A). A range of expression levels were observed among the ten lines, line GSSN 8-4 was chosen for further study as it displayed the highest level of expression. Production of the harpin_{Ea} protein in inoculated plants was confirmed by immuno-blot analysis.

RNA was isolated from inoculated plants over a 4 day interval to analyze *hrpN* gene expression. RNA was isolated from 1g of plant tissue as described by Carpenter et al., "Preparation of RNA, in Arabidopsis Protocols," (Martinez-Zapater, JM. and Salinas, J., eds.), Humana Press, Totowata, New Jersey, pp. 85-89 (1998). Twenty micro-gram samples were separated by formaldehyde-agarose gel electrophoresis and blotted onto Hybond N+ membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). Hybridizations and washing were performed according to Church et al., "Genomic Sequencing," Proc. Natl. Acad. Sci. USA 81:1991-1995 (1984), which is hereby incorporated by reference, using P^{32} labeled *hrpN* DNA as a probe.

The *Arabidopsis* lines GSSN 8-4 (test), Col-0 WT (wild type, control), and Col-0 EV (empty vector, control) were inoculated by drop inoculation with a conidiospore suspension (5×10^4 spores/ml) of *P. parasitica*. Plants were maintained in a growth chamber (16 hours of light, 18° C, 100% humidity) and were scored for infection ten days post inoculation. Nearly all (29 out of 30) 8-4 plants were free of

any signs of *P. parasitica* (Figure 4A). Trypan blue staining showed that growth of the oomycete was strongly inhibited in GSSN 8-4 plants. Extensive hyphal growth was evident in Col-0 WT and Col-0 EV plants (Figure 4B).

Plants were rated for disease severity based on the number of conidiophores per leaf. Nearly all GSSN 8-4 plants received a disease rating of 1 with only one being scored 3. The majority of the Col-0 WT and Col-0 EV plants were rated 5, the remainder were rated 4 (Figure 5).

This example demonstrates that pathogen inducible expression of the harpin$_{Ea}$ hypersensitive response elicitor of *Erwinia amylovora* in transgenic plants is a potentially useful strategy for engineering plants for disease resistance. Challenge with *Peronospora parasitica* resulted in accumulation of *hrpN* mRNA, production of harpin$_{Ea}$ protein, and resistance to *P. parasitica*. Upon challenge by *P. parasitica*, it is believed that the transgenic plants most likely mount a hypersensitive response at the site of inoculation, conferring resistance. Subsequently the plants may develop systemic resistance.

For the purposes of the present invention, the *gst1* promoter was most applicable to the *Arabidopsis/P. parasitica* pathosystem since it is well documented that transcription from *gst1* is activated by other oomycete pathogens (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). Additionally, it has been reported that *gst1* activation is stimulated by ascomycete, viral, and nematode infection and mycorrhization (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato gst1 Gene," Mol. Plant-Microbe Interact. 9:68-73 (1996), which is hereby incorporated by reference). Therefore, it is possible that both *gst1:hrpN* and *gst1*:signal sequence:*hrpN* constructs may also confer resistance against ascomycete, virus, and nematode infection, as well as mycorrhization.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

All of the references designated as being incorporated herein by reference are intended to be incorporated in their entirety unless specific portions thereof have been identified with particularity.

WHAT IS CLAIMED:

1. A chimeric gene comprising:

a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide,

a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and

a 3' regulatory region operably linked to the first DNA molecule.

2. The chimeric gene according to claim 1 further comprising:

a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

3. The chimeric gene according to claim 2, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.

4. The chimeric gene according to claim 3, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.

5. The chimeric gene according to claim 1, wherein the promoter is a *gst1* promoter.

6. The chimeric gene according to claim 1, wherein the *gst1* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.

7. The chimeric gene according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

8. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

9. The chimeric gene according to claim 8, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

10. The chimeric gene according to claim 9, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

11. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

12. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

13. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

14. The chimeric gene according to claim 13, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

15. The chimeric gene according to claim 14, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

16. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

17. The chimeric gene according to claim 16. wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.

18. The chimeric gene according to claim 17. wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.

19. The chimeric gene according to claim 7. wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

20. The chimeric gene according to claim 19. wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.

21. The chimeric gene according to claim 20. wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.

22. An expression system comprising a vector into which is inserted a chimeric gene according to claim 1.

23. A host cell comprising a chimeric gene according to claim 1.

24. The host cell according to claim 23, wherein the host cell is a bacterial cell or a plant cell.

25. The host cell according to claim 24, wherein the bacterial cell is an *Agrobacterium* cell.

26. The host cell according to claim 24, wherein the host cell is a plant cell.

27. The host cell according to claim 26, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

28. The host cell according to claim 26, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

29. The host cell according to claim 26, wherein the promoter is a *gst1* promoter.

30. A transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant comprising:

a chimeric gene according to claim 1, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

31. The transgenic plant according to claim 30, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

32. The transgenic plant according to claim 31, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.

33. The transgenic plant according to claim 32, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.

34. The transgenic plant according to claim 30, wherein the *gst1* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.

35. The transgenic plant according to claim 30, wherein the oomycete is a species of *Plasmopara, Phytophthora, Peronospora, Pseudoperonospora, Bremia, Sclerospora, Aphanomyces, Pythium*, or *Albugo*.

36. The transgenic plant according to claim 30, wherein the transgenic plant is selected from a group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

37. The transgenic plant according to claim 36, wherein the transgenic plant is a grape plant.

38. The transgenic plant according to claim 37, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.

39. The transgenic plant according to claim 36, wherein the transgenic plant is a tobacco plant.

40. The transgenic plant according to claim 39, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Pythium* spp., and *Phytophthora* spp.

41. The transgenic plant according to claim 30, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

42. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

43. The transgenic plant according to claim 42, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

44. The transgenic plant according to claim 44, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

45. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

46. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

47. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

48. The transgenic plant according to claim 47, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

49. The transgenic plant according to claim 48, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

50. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

51. The transgenic plant according to claim 50, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.

52. The transgenic plant according to claim 51, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.

53. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

54. The transgenic plant according to claim 53. wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.

55. The transgenic plant according to claim 54. wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.

56. The transgenic plant according to claim 30, wherein the chimeric gene is stably inserted into the genome of the transgenic plant.

57. A method of making a recombinant plant cell comprising:

transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.

58. A method of making a plant resistant to disease resulting from oomycete infection, the method comprising:

transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and

regenerating a plant from the transformed plant cell.

59. The method according to claim 58, wherein said transforming is performed under conditions effective to insert the chimeric gene into the genome of the plant cell.

60. The method according to claim 58, wherein said transforming is *Agrobacterium* mediated.

61. The method according to claim 58, wherein said transforming comprises:

propelling particles at the plant cell under conditions effective for the particles to penetrate into the cell interior and

introducing an expression vector comprising the chimeric gene into the plant cell interior.

62. The method according to claim 58, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

63. The method according to claim 58, wherein the promoter is a *gst1* promoter.

64. The method according to claim 58, wherein the oomycete is a species of *Plasmopara, Phytophthora, Peronospora, Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, or *Albugo*.

65. The method according to claim 58, wherein the transgenic plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

66. The method according to claim 65, wherein the transgenic plant is a grape plant.

67. The method according to claim 66, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.

68. The method according to claim 65, wherein the transgenic plant is a tobacco plant.

69. The method according to claim 68, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Phytophthora* spp., and *Pythium* spp.

70. The method according to claim 58, wherein the hypersensitive response elicitor protein or polypeptide derives from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

71. A transgenic plant seed obtained from the transgenic plant according to claim 30.

72. A transgenic plant scion or rootstock cultivar obtained from the transgenic plant according to claim 30.

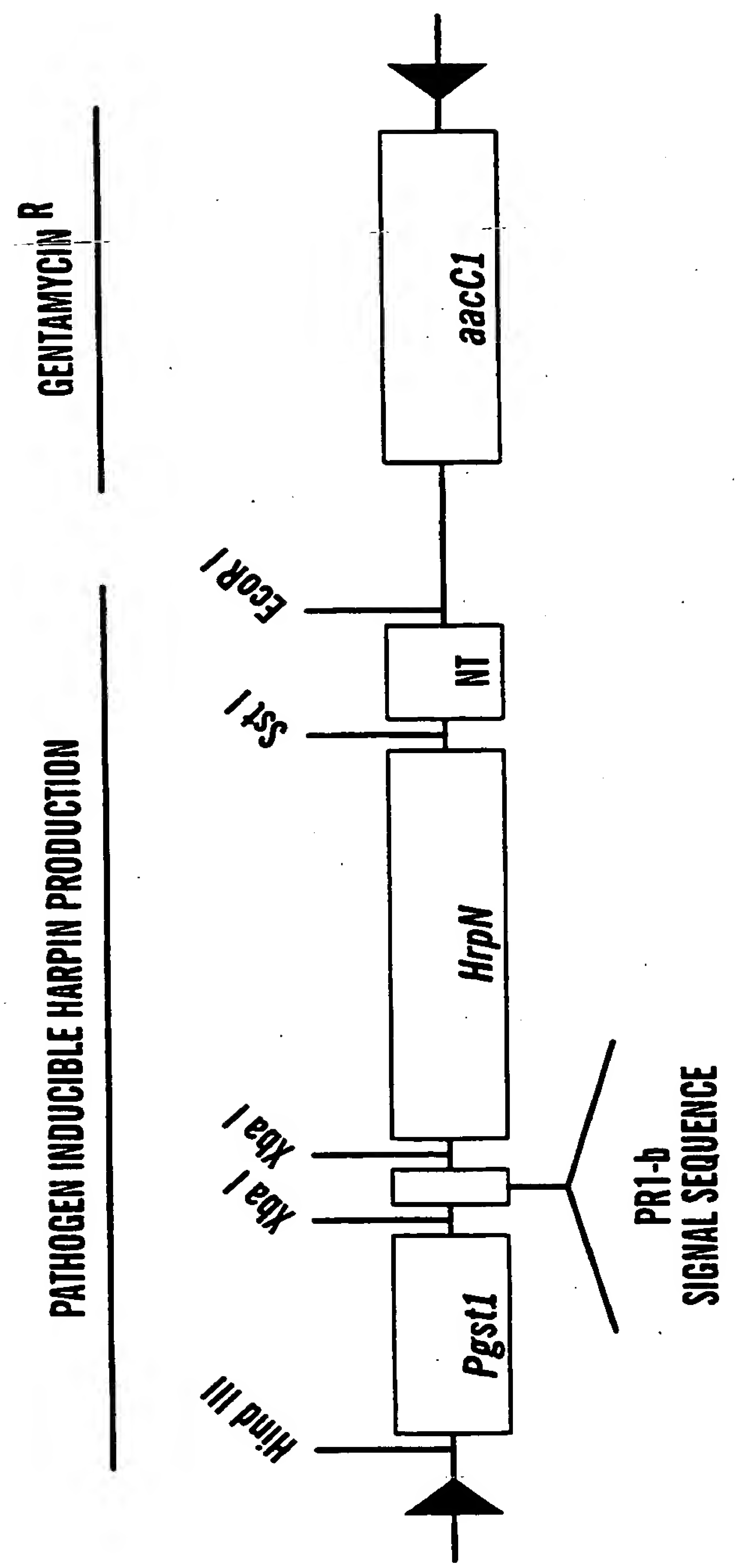
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FIG. 1

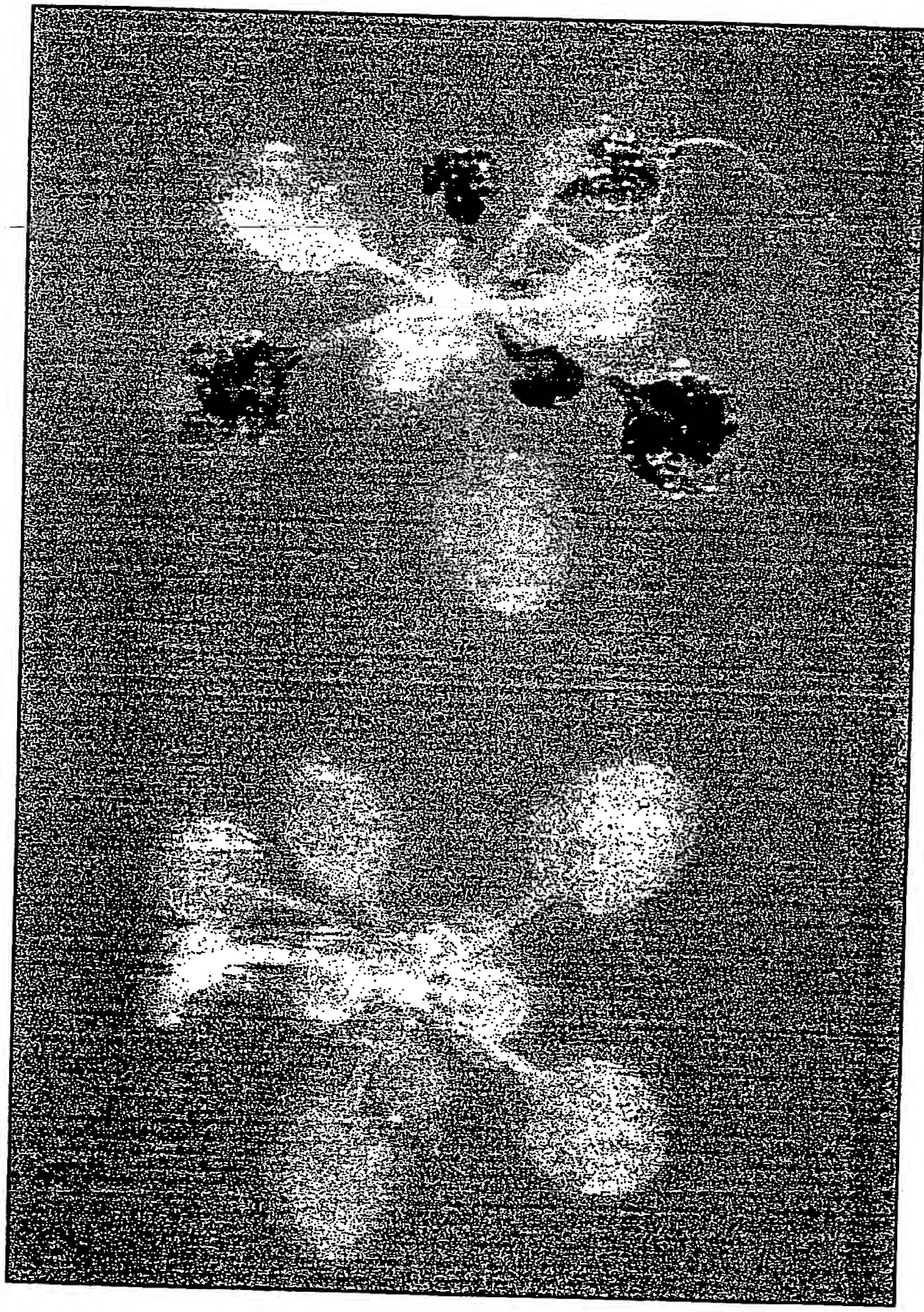


FIG. 2

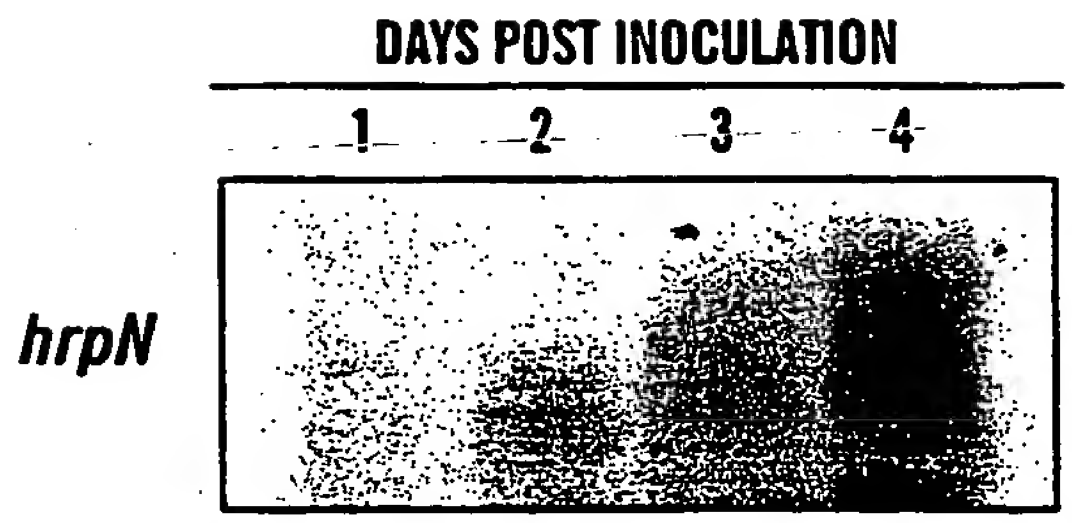


FIG. 3A

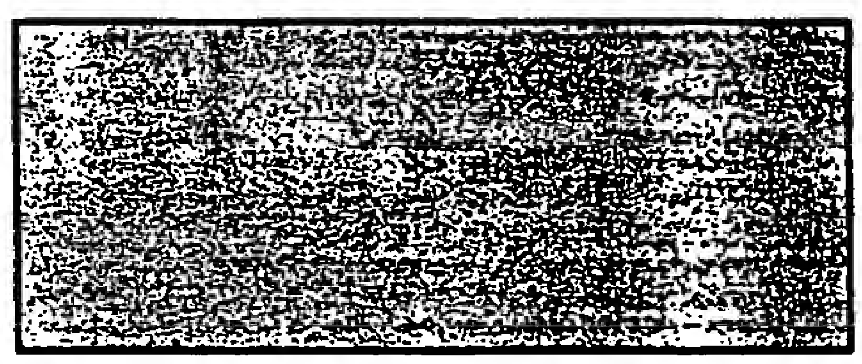


FIG. 3B

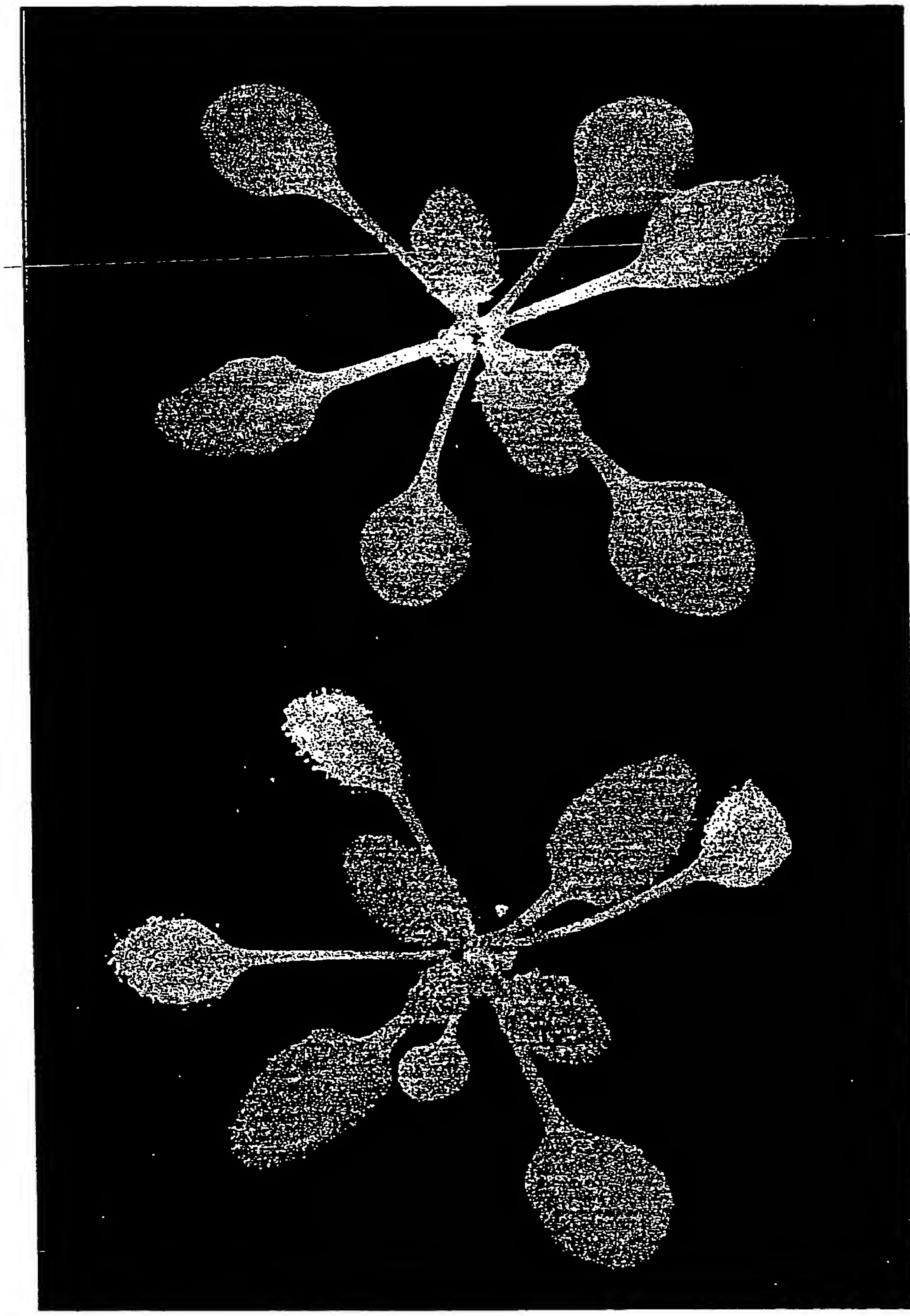


FIG. 4A

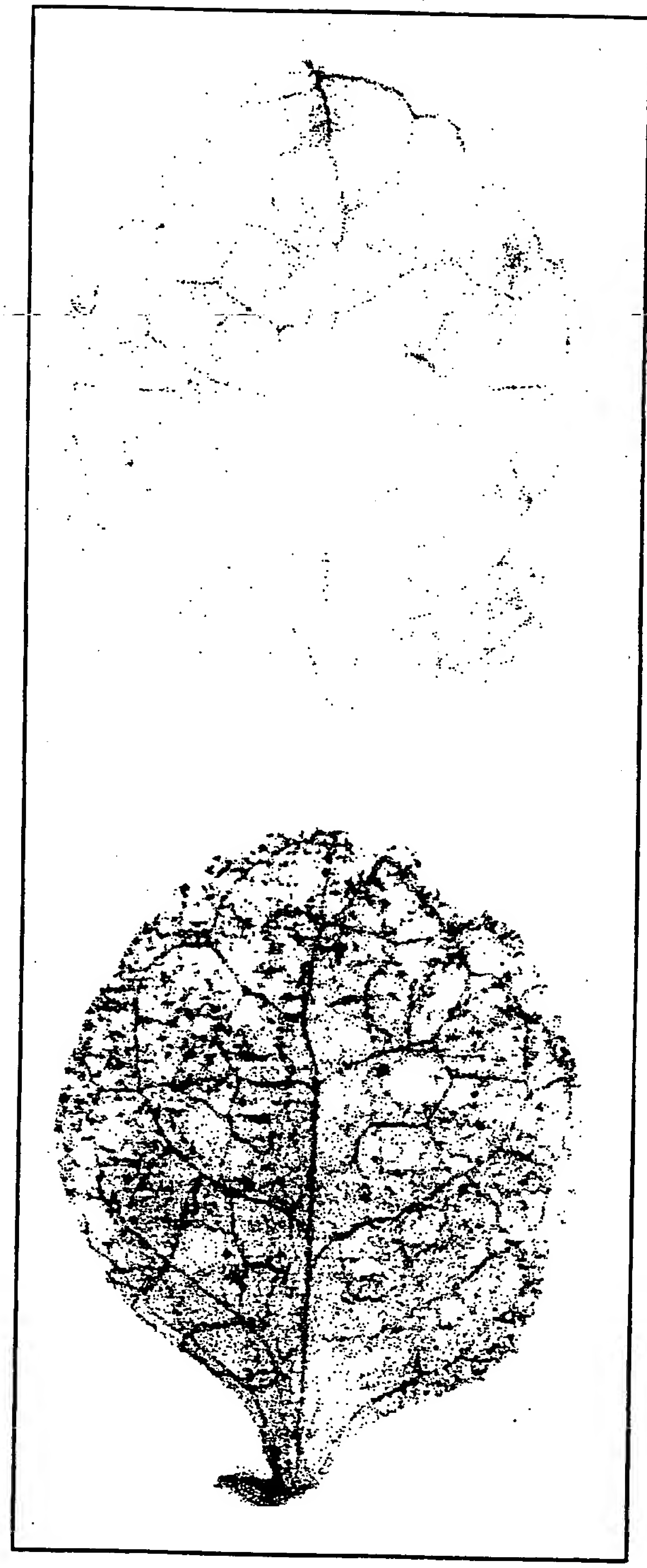
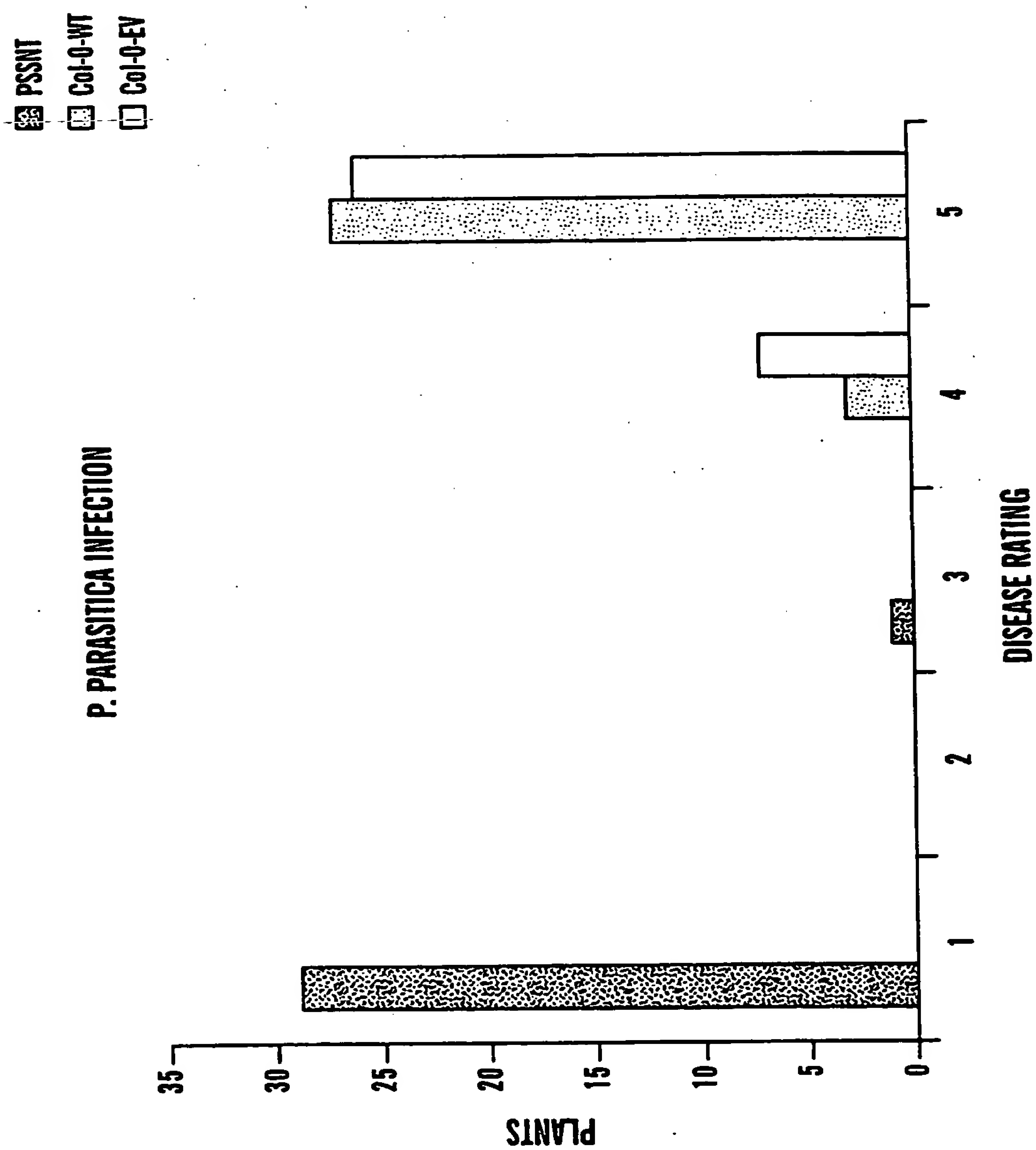


FIG. 4B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Col-0-EV
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2
3
4
5
DISEASE RATING

FIG. 5

SEQUENCE LISTING

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<212> PRT
<213> Pseudomonas solanacearum

<400> 7

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65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
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Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
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Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
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180 185 190

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210 215 220

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<213> Nicotiana tabacum

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/02579

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C12N 5/04; 15/09, 15/29, 15/31, 15/82; A01H 5/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS, WEST2.0
search terms: hypersensitive, elicitor, oomycete, gst1 promoter, transgenic plants , disease resistance, fungal pathogen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,859,332 A (STRITTMATTER et al) 12 January 1999 (12.01.99), see entire document, especially columns 3-5, 11-18, 23-26.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72
A	HART et al. Regulated Inactivation of Homologous Gene Expression in Transgenic Nicotiana sylvestris Plants Containing a Defense-related Tobacco Chitinase Gen. Mol. Gene. Genet. 1992, Vol. 235, No 2-3, pages 179-186, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72
Y	WEI et al. Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora. Science 03 July 1992, Vol. 257, pages 85-88, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 MAY 2001	14 JUN 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Joyce Bridgers for MEDINA A. IBRAHIM
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/02579

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a specified gst1 promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.
Group II, claim(s) 1-4, 22-26, 28, 31-33, 62, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a second DNA molecule encoding specific sequences of secretion signal polypeptides, a gst1 promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.
Group III, claim(s) 1, 7-21, 22-26, 27, 41-55, 70, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide of a specified sequence, a gst1 promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.
The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number
WO 01/70988 A2

(51) International Patent Classification[7]: C12N 15/29, 15/82, 15/11, 1/21, 5/10, A01H 5/00, G01N 33/68, A01N 65/00, 63/00

(21) International Application Number: PCT/US01/08728

(22) International Filing Date: 19 March 2001 (19.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/191,649 23 March 2000 (23.03.2000) US
60/250,710 1 December 2000 (01.12.2000) US

(71) Applicant: EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US).

(72) Inventors: SONG, Xiaoling; 14110 NE 179th Street, Apt. 75, Woodinville, WA 98072 (US). FAN, Hao; 19712 6th Drive S.E., Bothell, WA 98012 (US). WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US).

(74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 31051, Rochester, NY 14603-1051 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/70988 A2

(54) Title: RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF

(57) Abstract: The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules. Both the protein and nucleic acid can be used to identify agents targeting plant cells to enhance a plant's receptivity to treatment with a hypersensitive response elicitor and to directly impart plant growth enhancement as well as resistance against disease, insects, and stress.

RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF

This application claims benefit of U.S. Provisional Patent Application Serial Nos. 60/191,649, filed March 23, 2000 and 60/250,710, filed December 1, 2000.

FIELD OF THE INVENTION

The present invention relates to receptors for hypersensitive response elicitors and uses thereof.

BACKGROUND OF THE INVENTION

Plants have evolved a complex array of biochemical pathways that enable them to recognize and respond to environmental signals, including pathogen infection. There are two major types of interactions between a pathogen and plant -- compatible and incompatible. When a pathogen and a plant are compatible, disease generally occurs. If a pathogen and a plant are incompatible, the plant is usually resistant to that particular pathogen. In an incompatible interaction, a plant will restrict pathogen proliferation by causing localized necrosis, or death of tissues, to a small zone surrounding the site of infection. This reaction by the plant is defined as the hypersensitive response ("HR") (Kiraly, Z. "Defenses Triggered by the Invader: Hypersensitivity," Plant Disease: An Advanced Treatise 5:201-224 J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York (1980); (Klement "Hypersensitivity," Phytopathogenic Prokaryotes 2:149-177, M.S. Mount and G. H. Lacy, eds. Academic Press, New York (1982)). The localized cell death not only contains the infecting pathogen from spreading further but also leads to a systemic resistance preventing subsequent infections by other pathogens. Therefore, HR is a common form of plant resistance to diseases caused by bacteria, fungi, nematodes, and viruses.

A set of genes designated as *hrp* (Hypersensitive Response and Pathogenicity) is responsible for the elicitation of the HR by pathogenic bacteria, including *Erwinia* spp, *Pseudomonas* spp, *Xanthomonas* spp, and *Ralstonia solanacearum* (Willis et al. "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-

Microbe Interact. 4:132-138 (1991), Bonas, U. "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology, Vol. 192, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. J. L. Dangl, ed. Springer-Verlag, Berlin (1994); Alfano et al., "Bacterial Pathogens in Plants: Life Up Against the Wall," Plant Cell 8:1683-98 (1996). Typically, there are multiple *hrp* genes clustered in a 30-40 kb DNA. Mutation in any one of the *hrp* genes will result in the loss of bacterial pathogenicity in host plants and the HR in non-host plants. On the basis of genetic and biochemical characterization, the function of the *hrp* genes can be classified into three groups: 1) structural genes encoding extracellularly located HR elicitors, for example harpin of *Erwinia amylovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)); 2) secretion genes encoding a secretory apparatus for exporting HR elicitors and other proteins from the bacterial cytoplasm to the cell surface or extracellular space (Van Gijsegem et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993); He et al, "Pseudomonas syringae pv. Syringae $harpin_{pss}$: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255 (1993); Wei et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7985-67 (1993), Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), Galan et al., "Cross-talk between Bacterial Pathogens and their Host Cells," Ann. Rev. Cell Dev. Biol. 12:221-55 (1996); Bogdanove et al., "*Erwinia amylovora* Secretes Harpin via a Type III Pathway and Contains a Homolog of yopN of Yersinia," J. Bacteriol. 178:1720-30 (1996); Bogdanove et al., "Homology and Functional Similarity of a hrp-linked Pathogenicity Operon, *dspEF*, of *Erwinia amylovora* and the *avrE* locus of *Pseudomonas syringae* pathovar tomato," Proc Natl Acad Sci USA 95:1325-30 (1998)); and 3) regulatory genes that control the expression of *hrp* genes (Wei, Z. M., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); Wei et al., "*hrpL* Activates *Erwinia amylovora hrp* Genes in Response to

Environmental Stimuli," J. Bacteriol. 174:1875-82 (1995); Xiao et al., "A Single Promoter Sequence Recognized by a Newly Identified Alternate Sigma Factor Directs Expression of Pathogenicity and Host Range Determinants in *Pseudomonas syringae*," J. Bacterial 176:3089-91 (1994); Kim et al., "The hrpA and hrpC Operons of *Erwinia amylovora* Encode Components of a Type III Pathway that Secrets Harpin," J. Bacteriol 179:1690-97 (1997); Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-10 (1998); Wengelnik et al., "HrpG, A Key hrp Regulatory Protein of Xanthomonas campestris pv. Vesicatoria is Homologous to Two Component Response Regulators," Mol. Plant-Microbe Interact. 9:704-12 (1996)). Because of their role in interactions between plants and microbes, *hrp* genes have been a focus for bacterial pathogenicity and plant defense studies.

In addition to the local defense response, HR also activates the defense system in uninfected parts of the same plant. This results in a general systemic resistance to a secondary infection termed Systemic Acquired Resistance ("SAR") (Ross, R. F. "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," Virology 14:340-58 (1961); Malamy et al., "Salicylic Acid and Plant Disease Resistance," Plant J. 2:643-654 (1990)). SAR confers long-lasting systemic disease resistance against a broad spectrum of pathogens and is associated with the expression of a certain set of genes (Ward et al. "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," Plant Cell 3:1085-94 (1991)). SAR is an important component of the disease resistance of plants and has long been of interest, because the potential of inducing the plant to protect itself could significantly reduce or eliminate the need for chemical pesticides. SAR can be induced by biotic (microbes) and abiotic (chemical) agents (Gorlach et al. "Benzothiadiazole, a Novel Class of Inducers of Systemic Acquired Resistance, Activates Gene Expression and Disease Resistance in Wheat," Plant Cell 8:629-43 (1996)). Historically, weak virulent pathogens were used as a biotic inducing agent for SAR. Non-virulent plant growth promotion bacteria (PGPR) were also reported to be able to induce resistance of some plants against various diseases. Biotic agent-induced SAR has been the subject of much research, especially in the late 70s and early 80s. Only very limited success was achieved, however, due to: 1) inconsistency

of the performance of living organisms in different environmental conditions; 2) considerable concerns regarding the unpredictable consequences of the intentional introduction of weakly virulent pathogens into the environment; and 3) the technical complication of applying a living microorganism into a variety of environmental conditions. To overcome the limitations of using living organisms to induce SAR, scientists have long been looking for an HR elicitor derived from a pathogen for SAR induction. With the advancement of molecular biology, the first proteinaceous HR elicitor with broad host spectrum was isolated in 1992 from *Erwinia amylovora*, a pathogenic bacterium causing fire blight in apple and pear. The HR elicitor was named "harpin". It consists of 403 amino acids with a molecular weight about 40 kDa. The harpin protein is heat-stable and glycine-rich with no cysteine. The gene encoding the harpin protein is contained in a 1.3 kB DNA fragment located in the middle of the *hrp* gene cluster. Harpin is secreted into the extracellular space and is very sensitive to proteinase digestion. Since the first harpin was isolated from *Erwinia amylovora*, several harpin or harpin-like proteins have been isolated from other major groups of plant pathogenic bacteria. In addition to the harpin of *Erwinia amylovora*, the following harpin or harpin-like proteins have been isolated and characterized: HrpN of *Erwinia chrysanthemi*, *Erwinia carotovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science, 257:85 (1992)), and *Erwinia stewartii*; HrpZ of *Pseudomonas syringae* (He et al, "Pseudomonas syringae pv. Syringae harpin$_{pss}$: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255 (1993)), PopA of *Ralstonia solanacearum*, (Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)); HrpW of *Erwinia amylovora* (Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-10 (1998)), and *Pseudomonas syringae*. All of the currently described harpin or harpin-like proteins share common characteristics. They are heat-stable and glycine-rich proteins with no cysteine amino acid residue, are very sensitive to digestion by proteinases, and elicit the HR and induce resistance in many plants against many diseases. Based on their shared biochemical and

biophysical characteristics as well as biological functions, these HR elicitors from different pathogenic bacteria belong to a new protein family – i.e. the harpin protein family. The described characteristics, especially their ability to induce HR in a broad range of plants, distinguish the harpin protein family from other host specific proteinaceous HR elicitors, for example elicitins from *Phytophthora* spp (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," Plant Physiol 110:365-76 (1996)) or avirulence proteins (such as Avr9) from *Cladosporium fulvum*, which are only able to elicit the HR in a specific variety or species of a plant.

In nature, when certain bacterial infections occur, harpin protein is expressed and then secreted by the bacteria, signaling the plant to mount a defense against the infection. Harpin serves as a signal to activate plant defense and other physiological systems, which include SAR, growth enhancement, and resistance to certain insect damage.

The current understanding of critical plant molecules that may have a significant role in interacting with elicitors and then triggering a sequential signal transduction cascade is described as follows.

Interaction of Plant Resistance Genes (R) and Pathogen Avirulence Genes (avr)

The concept of gene-for-gene interaction is that "for each gene determining resistance (R gene) in the host, there is a corresponding gene determining avirulence in the pathogen (avr gene)". In this model, pathogen avirulence genes generate a specific ligand molecule, called an elicitor. Only plants carrying the matching resistance gene respond to this elicitor and invoke the HR. In the past few years, several disease-resistance, R genes, have been cloned and sequenced. It was expected that R genes might encode components involved in signal recognition or signal transduction pathways that ultimately lead to defense responses. The cloned R genes could be grouped into four classes: (1) cytoplasmic protein kinase; (2) protein kinases with an extracellular domain; (3) cytoplasmic proteins with a region of leucine-rich repeats and a nucleotide-binding site; and (4) proteins with a region of

leucine-rich repeats that appear to encode extracellular proteins. (Review in Bent, A.F. "Plant Disease Resistance Genes: Function Meets Structure," Plant Cell 8:1757-71 (1996); Baker B., et al., "Signaling in Plant-Microbe Interactions," Science 276:726-33 (1997)). The first R gene cloned, Pto, encodes a serine/threonine protein kinase. The protein product of Pto directly interacts with the cognate avirulence gene protein, AvrPro, which has been demonstrated in a yeast two-hybrid system. It was shown that only co-existence of both AvrPro and Pto proteins could elicit HR in plants (Tang et al., "Initiation of Plant Disease Resistance by Physical Interaction of AvrPto and Pto kinase," Science 274:2060-63 (1996); Scofield et al., "Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato," Science 274:2063-65 (1996); Zhou et al., "The Pto kinase Conferring Resistance to Tomato Bacterial Speck Disease Interacts with Proteins that Bind a cis-element of Pathogenesis-related Genes," EMBO J. 16:3207-18 (1997)). The results from cloned R genes support the view that plant-pathogen interactions involve protein-protein interactions. Syringolide, a water-soluble, low-molecular-weight elicitor, triggers a defense response in soybean cultivars carrying the Rpg4 disease-resistance gene. A 34-KDa protein has been isolated from soybean and is considered to be the physiological active syringolide receptor (Ji et al., "Characterization of a 34-kDa Soybean Binding Protein for the syringolide Elicitors," Proc. Natl. Acad. Sci. USA 95:3306-11 (1998)).

Putative Binding Factor of Elicitin

Elicitins are a family of small proteins secreted by *Phytophthora* species that have a high degree of homology. Pure elicitins alone can cause a hypersensitive response, a local cell death, and trigger systemic acquired resistance in tobacco and other plants (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," Plant Physiol 110:365-76 (1996)). However, the spectrum of HR elicitation and induced systemic resistance in plants is much narrower than that achieved by harpin family elicitors. Like harpin, elicitins induce a series of metabolic events in tobacco cells, including the accumulation of phytoalexins, ethylene

production, transmembrane electrolyte leakage, H_2O_2 accumulation, and expression of plant defense related genes (Yu L, et al., "Elicitins from Phytophthora and Basic Resistance in Tobacco," Proc. Natl. (1995); Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and Nonspecific Disease Resistance," The Plant Cell 11:223-35 (1999)). A putative receptor-like binding factor has been identified in tobacco plasma membrane, which has a specific high-affinity to the crytogein, one member of the elicitin family (Wendehenne, et al., "Evidence for Specific, High-Affinity Binding Sites for a Proteinaceous Elicitor in Tobacco Plasma Membrane," FEBS Letters 374:203-207 (1995)). Recently, it was found that 2 basic elicitins (i.e. cryptogein and cinnamomin) and two acidic elicitins (i.e. capsicein and parasiticein) were able to interact with the same binding sites on tobacco plasma membranes (Bourque et al., "Comparison of Binding Properties and Early Biological Effects of Elicitins in Tobacco Cells," Plant Physiol. 118:1317-26 (1998)). However, the gene of the receptor-like factor has not been isolated.

Putative Binding Factor of Glycoprotein Elicitors

A 42 kDa glycoprotein elicitor has been isolated from *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991)). An oligopeptide of 13 amino acids within the glycoprotein ("Pep-13") was able to induce a response in plants like that achieved by the full glycoprotein. A high affinity-binding pattern has been observed in parsley microsomal membranes with an isotope labeled oligopeptide. There are estimated to be about 1600 to 2900 binding sites per cell with evidence indicating that a low abundant protein receptor of the Pep-13 is localized in the plasma membrane (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Responses," Cell 78:449-60 (1994)).

Harpin Protein Binding Factors

Harpin proteins, which elicit HR in a variety of different nonhost plants, have been isolated from plant pathogens (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)). A family of harpin proteins has been identified from plant bacterial pathogens. All of them have similar biological activities. It is well documented that harpin protein can induce plants to produce active oxygen, change ion flux, lead to local cell death, and induce systemic acquired resistance ("SAR") (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); He et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993); Baker, C.J., et al., "Harpin, an Elicitor of the Hypersensitive Response in Tobacco Caused by *Erwinia amylovora*, Elicits Active Oxygen Production in Suspension Cells," Plant Physiol. 102:1341-44 (1993)). No harpin protein binding factor has been isolated so far. It was reported that an amphipathic protein, named HRAP, isolated from sweet pepper could dissociate harpin$_{pss}$ in multimeric form (*hrpZ* from *Pseduomonas syringae*). The biological activity of the HRAP is believed to be its ability to intensify harpin$_{pss}$-mediated hypersensitive response. HRAP protein does not bind to harpin$_{pss}$ directly (Chen et al., "An Amphipathic Protein from Sweet Pepper can Dissociate Harpin$_{pss}$ Multimeric Forms and Intensify the Harpin$_{pss}$ -Mediated Hypersensitive Response," Physiological & Molecular Pathology 52:139-49 (1998)). Using a fluorochrome tagged antibody to harpin to examine the interaction of harpin$_{pss}$ and tobacco suspension cells, it was found that harpin$_{pss}$ interacted with the cultured cells, but not with protoplasts with the cell walls being digested and removed. It was interpreted that harpin$_{pss}$ was localized in the outer portion of the plant cell, probably on the cell well. However, it was not ruled out that the binding factor was located on the plasma membrane.

The present invention seeks to identify receptors for hypersensitive response elicitor proteins or polypeptides and uses of such receptors.

SUMMARY OF THE INVENTION

The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules.

The protein of the present invention can be used with a method of identifying agents targeting plant cells by forming a reaction mixture including the protein and a candidate agent, evaluating the reaction mixture for binding between the protein and the candidate agent, and identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

The nucleic acid molecule of the present invention can be used in a method of identifying agents targeting plant cells by forming a reaction mixture including a cell transformed with the nucleic acid molecule of the present invention and a candidate agent, evaluating the reaction mixture for binding between protein produced by the host cell and candidate agent, and identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

Another aspect of the present invention relates to a method of enhancing a plant's receptivity to treatment with hypersensitive response elicitors by providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule of the present invention.

The present invention is also directed to a method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants by providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule encoding a receptor in accordance with the present invention.

The discovery of the present invention has great significance. This putative receptor protein can be used as a novel way to screen for new inducers of plant resistance against insect, disease, and stress, and of growth enhancement. This protein is the first step toward the understanding of the harpin induced signal transduction pathway in plants. Further studies of this pathway will provide more

possible targets for new plant vaccine and growth enhancement products development. In addition, this protein can serve as an anchor providing a new way to target anything to the plant cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a yeast two-hybrid screening with the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) and a schematic representation of the interaction between harpin and a cDNA encoded polypeptide. Harpin is fused to LexA protein which contains a DNA binding domain ("BD"). The cDNA encoded polypeptide is fused to the GAL4 transcription activation domain ("AD"). This interaction targets the activation domain to two different LexA-dependent promoters with consequent activation of the transcription of the *HIS3* and *lacZ* reporter genes.

Figures 2A-B show that the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) is a good yeast two-hybrid bait. Reporter genes were not expressed in yeast strain L40 containing plasmids expressing the LexA - harpin fusion in combination with plasmids expressing the GAL4 activation domain alone, or fused to unrelated protein. Therefore, harpin is not autoactive in this yeast two-hybrid system. In addition, reporter genes were not expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-harpin fusion in combination with plasmids expressing LexA alone, or fused to unrelated protein. Figure 2A shows a β-galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 2B shows a synthetic minimal ("SD") media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figures 3A-B show the interaction between HrBP1 (hypersensitive response elicitor binding protein 1) and a hypersensitive response elicitor (i.e. harpin) is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-HrBP1 fusion in combination with plasmids expressing LexA fused to hypersensitive response elicitor (i.e. harpin), but were not expressed in combination with LexA alone, or LexA fused to unrelated proteins.

Figure 3A is a β-galactosidase assay where the blue color indicates the expression of *lacZ* reporter gene. Figure 3B is an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figures 4A-B show the interaction of HrBP1 and a hypersensitive response elicitor (i.e. harpin) in another orientation. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the LexA - HrBP1 fusion in combination with plasmids expressing GAL4 activation domain fused to harpin, but were not expressed in combination with GAL4 activation domain alone, or GAL4 activation domain fused to unrelated proteins. Therefore, interaction between harpin and HrBP1 is specific. Figure 4A shows a β-galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 4B shows an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figure 5 shows the gene structure of HrBP1 and a schematic representation of the exons and introns of the HrBP1 gene. When comparing the HrBP1 cDNA sequence with the *Arabidopsis thaliana* genomic DNA sequence published in a public database, four exons and three introns were discovered.

Figure 6 shows a Northern blot using RNA probe complementary to bases 651-855 of HrBP1 coding region (SEQ. ID. No. 9).

Figures 7A-B show that the interaction between rHrBP1 (R6) and harpin is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-rHrBP1 fusion in combination with plasmids expressing LexA fused to harpin or harpin 137-180 amino acids, but were not expressed in combination with LexA alone, LexA fused to unrelated proteins, or fused to harpin 210-403 amino acids. Figure 7A shows a β-galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 7B shows a SD media plate, which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figure 8 shows the constructs used to "knockout" HrBP1 gene in Arabidopsis.

Figure 9A-C show a *Pseudomonas syringae* p.v. tomato DC3000 assay on wild type and HrBP1 "knockout" transgenic Arabidopsis plants. Figure 9A is a picture taken 7 days after *P. syringae* inoculation. In Figure 9B, leaf disks were harvested. Bacteria were extracted from leaf disks and plated onto King's B agar plate containing 100μg/ml rifampicin. Figure 9C shows the bacteria count from plates in Figure 9B. This signifies an anti-sense line and d refers to a double-stranded RNA line.

Figure 10 shows the construct used to overexpress HrBP1 in tobacco.

Figure 11A-B show the height of wild type and HrBP1 overexpressing tobacco plants 52 days after they were transferred to soil. Figure 11A is a picture taken 52 days after plants were transferred to soil. Figure 11B shows average height of 8 plants per line.

Figure 12A-B show a TMV assay results on wild type and HrBP1 overexpressing tobacco plants. Figure 12A is a picture taken 3 days after TMV inoculation. Figure 12B shows the average virus lesion diameter from 5 plants per line 3 days after TMV inoculation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated receptors for hypersensitive response elicitor proteins or polypeptides. Also disclosed are DNA molecules encoding such receptors as well as expression systems, host cells, and plants containing such molecules. Uses of the receptors themselves and the DNA molecules encoding them are disclosed. The receptor for a hypersensitive response elicitor from a plant pathogen can be from a monocot or a dicot.

One example of such a receptor is that found in *Arabidopsis thaliana* which has the amino acid sequence of SEQ. ID. No. 1 as follows:

```
Met Ala Thr Ser Ser Thr Phe Ser Ser Leu Leu Pro Ser Pro Pro Ala
1               5                   10                  15

L u Leu Ser Asp His Arg Ser Pro Pro Pro Ser Ile Arg Tyr Ser Phe
            20                  25                  30

S r Pro Leu Thr Thr Pro Lys Ser Ser Arg Leu Gly Phe Thr Val Pro
        35                  40                  45
```

```
Glu Lys Arg Asn Leu Ala Ala Asn Ser Ser Leu Val Glu Val Ser Ile
    50                  55                  60

Gly Gly Glu Ser Asp Pro Pro Pro Ser Ser Ser Gly Ser Gly Gly Asp
65                  70                  75                  80

Asp Lys Gln Ile Ala Leu Leu Lys Leu Lys Leu Leu Ser Val Val Ser
                85                  90                  95

Gly Leu Asn Arg Gly Leu Val Ala Ser Val Asp Asp Leu Glu Arg Ala
            100                 105                 110

Glu Val Ala Ala Lys Glu Leu Glu Thr Ala Gly Gly Pro Val Asp Leu
        115                 120                 125

Thr Asp Asp Leu Asp Lys Leu Gln Gly Lys Trp Arg Leu Leu Tyr Ser
    130                 135                 140

Ser Ala Phe Ser Ser Arg Ser Leu Gly Gly Ser Arg Pro Gly Leu Pro
145                 150                 155                 160

Thr Gly Arg Leu Ile Pro Val Thr Leu Gly Gln Val Phe Gln Arg Ile
                165                 170                 175

Asp Val Phe Ser Lys Asp Phe Asp Asn Ile Ala Glu Val Glu Leu Gly
            180                 185                 190

Ala Pro Trp Pro Phe Pro Pro Leu Glu Ala Thr Ala Thr Leu Ala His
        195                 200                 205

Lys Phe Glu Leu Leu Gly Thr Cys Lys Ile Lys Ile Thr Phe Glu Lys
    210                 215                 220

Thr Thr Val Lys Thr Ser Gly Asn Leu Ser Gln Ile Pro Pro Phe Asp
225                 230                 235                 240

Ile Pro Arg Leu Pro Asp Ser Phe Arg Pro Ser Ser Asn Pro Gly Thr
                245                 250                 255

Gly Asp Phe Glu Val Thr Tyr Val Asp Asp Thr Met Arg Ile Thr Arg
            260                 265                 270

Gly Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ala
        275                 280
```

This protein, known as HrBP1p, is encoded by a cDNA molecule having SEQ. ID. No. 2 as follows:

```
tttttccttc tcaacaatgg cgacttcttc tactttctcg tcactactac cttcaccacc 60
agctcttctt tccgaccacc gttctcctcc accatccatc agatactcct tttctccctt 120
aactactcca aaatcgtctc gtttgggttt cactgtaccg gagaagagaa acctcgctgc 180
taattcgtct ctcgttgaag tatccattgg cggagaaagt gacccaccac catcatcatc 240
tggatcagga ggagacgaca agcaaattgc attactcaaa ctcaaattac ttagtgtagt 300
ttcgggatta aacagaggac ttgtggcgag tgttgatgat ttagaaagag ctgaagtggc 360
tgctaaagaa cttgaaactg ctgggggacc ggttgattta accgatgatc ttgataagct 420
tcaagggaaa tggaggctgt tgtatagtag tgcgttctct tctcggtctt taggtggtag 480
ccgtcctggt ctacctactg gacgtttgat ccctgttact cttggccagg tgtttcaacg 540
```

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gattgatgtg tttagcaaag atttgataa catagcagag gtggaattag gagccccttg 600
gccatttccg ccattagaag ccactgcgac attggcacac aagtttgaac tcttaggcac 660
ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgtcgca 720
gattcctccg tttgatatcc cgaggcttcc cgacagtttc agaccatcgt caaaccctgg 780
aactggggat ttcgaagtta cctatgttga tgataccatg cgcataactc gcggggacag 840
aggtgaactt agggtattcg tcattgctta attctcaaag ctttgacatg taaagataaa 900
taaatacttt ctgcttgatg cagtctcatg agttttgtac aaatcatgtg aacatataaa 960
tgcgctttat aagtaaatga gtgtcttgtt caatgaatca                       1000
```

The genomic DNA molecule containing the receptor encoding cDNA molecule of SEQ. ID. No. 2 has SEQ. ID. No. 3 as follows:

```
aattagaaaa attaacaacc aacatctagt tagaatattt aatttgcacc aatgtcttcg 60
agtatagtga aaaaaataga agatcgaata tcgaatagta cgtatagaat catctagatc 120
cattcgaact aacgtctact tttcttttcc agcattaaca tgtagcttgt cattagcatt 180
tacatgttgc aaataacaca aattgggaaa ttgaaagact aaaaaacctt gtacagcaga 240
tggtttaaca cgtggattca tggacacaaa cagaaaacgg cagaactaag cacaaaaacg 300
tcaactaagc atatcaaagc ttttaatgca agcctaatat aaacacaagt ggttatccat 360
aatctgttct taatctcttg cagtagttat cttttcatta ttcgcaattc gcaattctat 420
attcttatat ttcaacttgt tcttcttcca aattgtaatt atatctacat cgtcttagct 480
tgaccattat agctccagta ccaagttctc ttcttaactt taatatcagc tactattctc 540
atactgtaaa tatcttttgt tcaccaaaca tatatttcga accaaactgc taaaagctta 600
tcataaattg cagttctagc cacacaattt tgcagttcca accattaaat gccacaaaat 660
ttggacgatt tcttaagaca agaagaacat agcaaccaaa ccttattgat taaatatgaa 720
atgtctccat aaaactggga gatttcccca aataaagaga acacggcaaa tgttcacgta 780
atctccaaga tgaatgttta atttttctt tcagaaaaaa acaaaaaaac ttaactcaat 840
atagacaact agaatggata ccaactaagc aaaagaaatt caaaagacaa atatatattg 900
gatatgaagt tacattattt tcaaacttta tatactacta aaagcctaaa aatttgttct 960
aaaatgatat ccaaataaat ggaaggcatg aatgtcatat gactaaaaga gaaaaacaca 1020
cctgtatata agtattggat catgctgcct ccgagtgaca aaacatacga tgtgggtctt 1080
tattgggcca tacttaaatg gaaaaaggag aaaaaaaatt gggcaatgtc tatggtcgaa 1140
atttatatgt tttacatcaa taaaatcaat atttaatttt atatatgtgg gtcttaatct 1200
agtattatct acatagatta aaatcaaagt actgcatatg gtccataata atacaaccaa 1260
agcaaattaa aattttgtgg cacaaaacga catcatttta ctcagaaagt aatatgcaat 1320
ttcgtttacg cacacacgta tacgcgctaa taacccgtgg tgcttctcaa atcacataat 1380
aattaaagtc ttcttcttct tcttcttctc tacaaattat ctcactctct tcgttttttt 1440
ttccttctca acaatggcga cttcttctac tttctcgtca ctactacctt caccaccagc 1500
tcttctttcc gaccaccgtt ctcctccacc atccatcaga tactcctttt ctcccttaac 1560
tactccaaaa tcgtctcgtt tgggtttcac tgtaccggag aagagaaacc tcgctgctaa 1620
ttcgtctctc gttgaagtat ccattggcgg agaaagtgac ccaccaccat catcatctgg 1680
atcaggagga gacgacaagc aaattgcatt actcaaactc aaattacttg tgagtctgat 1740
tcaaaccaat cggtgaaatt ataagaaatt ggtttcgttt ctttggaatt agggtttata 1800
ttactgttaa gattcgatta tagagtgaat tttgggaaga tttttcagat ttgatttgtg 1860
```

atgtgttgtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacaga ggacttgtgg 1920
cgagtgttga tgatttagaa agagctgaag tggctgctaa agaacttgaa actgctgggg 1980
gaccggttga tttaaccgat gatcttgata agcttcaagg gaaatggagg ctgttgtata 2040
gtagtgcgtt ctcttctcgg tctttaggtg gtagccgtcc tggtctacct actggacgtt 2100
tgatccctgt tactcttggc caggtaattc ttgaatcatt gctctgtttt tacccgtcaa 2160
gattcggttt ttcgggtaag ttgttgagga gtttatgtgc atggtctagt ctatcatcaa 2220
tagtcttgct tgagtttgaa tggggctgag ctaagaatct agctttctga ggttacaatt 2280
tggtaatgtc atcttatact cgaaagcaaa ctttttctta tattgtcaag tttatgtgta 2340
cggtctggtc tatcattggt agtctttgtt gagcttgaat ggtgaggagc ttagaatcta 2400
gcaatgtcat ctactcctta atcatttttt tctatattgc caagtttatg tgtacggtct 2460
tagtcaatca tctttattct tggttgagtt tgaatggtga tgagcttaga atctagcttt 2520
ctttggttta aatttggcaa agaaccatac ctgaatcggt agaaagcaaa cttctttaat 2580
attatctctt gtttctgaat cattaaaaca ggtgtttcaa cggattgatg tgtttagcaa 2640
agattttgat aacatagcag aggtggaatt aggagcccct tggccatttc cgccattaga 2700
agccactgcg acattggcac acaagtttga actcttaggt ttgcatttcc ctttctctca 2760
ttaagtttat cgaattgtgt aagagcaaaa taacttatct gtatctttga catttatggg 2820
gaaaacaggc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcggg 2880
aaacttgtcg cagattcctc cgtttgatat cccgaggctt cccgacagtt tcagaccatc 2940
gtcaaaccct ggaactgggg atttcgaagt tacctatgtt gatgatacca tgcgcataac 3000
tcgcggggac agaggtgaac ttagggtatt cgtcattgct taattctcaa agctttgaca 3060
tgtaaagata aataaatact ttctgcttga tgcagtctca tgagttttgt acaaatcatg 3120
tgaacatata aatgcgcttt ataagtaaat gagtgtcttg ttcaatgaat catatgaaag 3180
aatttgtatg actcagaaaa ttggacaatg atatagacct tccaaatttt gcaccctcta 3240
atgtgagata ttagtgattt tttcttaggt tggtagagag aacggattgg caaaaaaata 3300
tcgaaggtca atgattaaca gcaaaaccat atcttgatga ttcaaaatat agagttaaca 3360
agcaaagatg agacaatctt atacgagaga gctaaaacaa atggattcca aatccagcaa 3420
gtacaaaaat cgcagaaaat aagatgaaac caacttaaaa cagagatgtt ccctttccct 3480
tcttgtcacc accgatctcg aaatgcttgc acctctgaaa taaacaacaa accaacacaa 3540
tgtaagcaaa ttaccaagtt acaaatccgg tataatgaac tgatctatgt tctatgcacc 3600
ttgataggac gctgcgaaaa gtgcttgcag ctttgacact gaagcctcaa aacaatcttc 3660
ttcgtggtct tagcctgtta acaagattca caagatgtat ctcagtccaa aactgagact 3720
attggaatgt ctgtttcctc acagctcact tccaaaattc tactataaat ggttccttaa 3780
aactacctca tttcaactaa ctagacctaa ttcaaactga aaaaacaatc aatgcatgat 3840
aatcaatgtt accttttttgt ggaagacagg cttagtctga ccaccataac cagattgttt 3900
acggtcataa cgacgctttc cttgagcagc aagactgtct ttacccttct tgtattgggt 3960
aaccttgtgc aaagtatgct ttttgcattc cttgttctta cagtaagtgt tctttgtctt 4020
tggaatgttc accttcaaaa ttcataaaat caaaaatgaa tcactcacac acatacaaaa 4080
tcaagagact tttaaggtta atcaaaatac aaacatcatt tagattgaaa acttttatga 4140
tagatctgaa aaacaataca ataaatcaat caaccatgta ttgttgttct tcaaagtcaa 4200
cgaactttac aaattccaaa atcacatcga aagagaagaa acaatttacc attttcgcgt 4260

Another example of a receptor in accordance with the present invention is that found in rice which has a partial amino acid sequence of SEQ. ID. No. 4 as follows:

```
Val Ala Ala Leu Lys Val Lys Leu Leu Ser Ala Val Ser Gly Leu Asn
 1               5                  10                  15

Arg Gly Leu Ala Gly Ser Gln Glu Asp Leu Asp Arg Ala Asp Ala Ala
            20                  25                  30

Ala Arg Glu Leu Glu Ala Ala Ala Gly Gly Gly Pro Val Asp Leu Glu
        35                  40                  45

Arg Asp Val Asp Lys Leu Gln Gly Arg Trp Arg Leu Val Tyr Ser Ser
    50                  55                  60

Ala Phe Ser Ser Arg Thr Leu Gly Gly Ser Arg Pro Gly Pro Pro Thr
65                  70                  75                  80

Gly Arg Leu Leu Pro Ile Thr Leu Gly Gln Val Phe Gln Arg Ile Asp
                85                  90                  95

Val Val Ser Lys Asp Phe Asp Asn Ile Val Asp Val Glu Leu Gly Ala
            100                 105                 110

Pro Trp Pro Leu Pro Pro Val Glu Leu Thr Ala Thr Leu Ala His Lys
        115                 120                 125

Phe Glu Ile Ile Gly Thr Ser Ser Ile Lys Ile Thr Phe Asp Lys Thr
    130                 135                 140

Thr Val Lys Thr Lys Gly Asn Leu Ser Gln Leu Pro Pro Leu Glu Val
145                 150                 155                 160

Pro Arg Ile Pro Asp Asn Leu Arg Pro Pro Ser Asn Thr Gly Ser Gly
                165                 170                 175

Glu Phe Glu Val Thr Tyr Leu Asp Gly Asp Thr Arg Ile Thr Arg Gly
            180                 185                 190

Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ser
        195                 200
```

This protein, known as R6p, is encoded by a cDNA molecule which has a partial sequence corresponding to SEQ. ID. No. 5 as follows:

```
cgtggctgcg ctcaaagtca agcttctgag cgcggtgtcc gggctgaacc gcggcctcgc 60
ggggagccag gaggatcttg accgcgccga cgcggcggcg cgggagctcg aggcggcggc 120
gggtggcggc cccgtcgacc tggagaggga cgtggacaag ctgcaggggc ggtggaggct 180
ggtgtacagc agcgcgttct cgtcgcggac gctcggcggc agccgccccg gcccgcccac 240
cggccgcctc ctccccatca ccctcgggca ggtgtttcag aggatcgatg ttgtcagcaa 300
ggacttcgac aacatcgtcg atgtcgagct cggcgcgcca tggccgctgc cgccggtgga 360
gctgacggcg accctggctc acaagtttga gatcatcggc acctcgagca taaagatcac 420
attcgacaag acgacggtga agacgaaggg gaacctgtcc cagctgccgc cgctggaggt 480
ccctcgcatc ccggacaacc tccggccgcc gtccaacacc ggcagcggcg agttcgaggt 540
gacctacctc gacggcgaca cccgcatcac ccgcggggac agaggggagc tcagggtgtt 600
```

```
cgtcatctcg tga                                                     613
```

Hypersensitive response elicitors recognized by the receptors of the present invention are able to elicit local necrosis in plant tissue contacted by the elicitor.

Examples of suitable bacterial sources of hypersensitive response elicitor polypeptides or proteins include *Erwinia*, *Pseudomonas*, and *Xanthamonas* species (e.g., the following bacteria: *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* is disclosed in U.S. Patent No. 5,850,015 and U.S. Patent No. 6,001,959, which are hereby incorporated by reference. This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has a glycine content of greater than 21% and contains substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in U.S. Patent No. 5,849,868 to Beer and Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* and its encoding

DNA molecule is found in U.S. Patent Nos. 5,708,139 and 5,858,786 and He et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference. This protein has 344 amino acids, a molecular weight of 33.2 kDa, and a pI of 4.16, is heat stable and glycine rich (20.6%).

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has a partial amino acid sequence corresponding to SEQ. ID. No. 6 as follows:

```
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1               5                  10                  15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
            20                  25
```

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris pv. pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It has the amino acid sequence of SEQ. ID. No. 7 as follows:

```
Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr
 1               5                  10                  15

Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro
            20                  25                  30

Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile
        35                  40                  45
```

```
Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys
    50                  55                  60

Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu
65                  70                  75                  80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly
                85                  90                  95

Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly
            100                 105                 110

Asn Ala
```

This amino acid sequence is encoded by the nucleotide sequence of SEQ. ID. No. 8 as follows:

```
atggactcta tcggaaacaa cttttcgaat atcggcaacc tgcagacgat gggcatcggg 60
cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120
gatcagttgc tcgccatgtt catcatgatg atgctgcaac agagccaggg cagcgatgca 180
aatcaggagt gtggcaacga acaaccgcag aacggtcaac aggaaggcct gagtccgttg 240
acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300
ggcggtggcg gttcggtcaa cagcagcctg ggcggcaacg cc                    342
```

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. This protein has 356 amino acids, a molecular weight of 35.6 kDa, and a pI of 5.82 and is heat stable and glycine rich (21.3%).

The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma,* and *Phytophora citrophthora* are

described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference. These hypersensitive response elicitors from *Phytophthora* are called elicitins. All known elicitins have 98 amino acids and show >66% sequence identity. They can be classified into two groups, the basic elicitins and the acidic elicitins, based on the physicochemical properties. This classification also corresponds to differences in the elicitins' ability to elicit HR-like symptoms. Basic elicitins are 100 times more effective than the acidic ones in causing leaf necrosis on tobacco plants.

The hypersensitive response elicitor from Gram positive bacteria like *Clavibacter michiganesis* is described in WO 99/11133, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response using conditions under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Turning again to the receptor of the present invention for such hypersensitive response elicitors, fragments of the above receptor protein are encompassed by the method of the present invention. In addition, fragments of full length receptor proteins from other plants can also be utilized.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known receptor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for receptor activity according to the procedure described above.

As an alternative, fragments of a receptor protein can be produced by digestion of a full-length receptor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave receptor proteins at different sites based on the amino acid sequence of the receptor protein. Some of the fragments that result from proteolysis may be active receptors.

In another approach, based on knowledge of the primary structure of the receptor protein, fragments of the receptor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the receptor being produced. Alternatively, subjecting a full length receptor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a tag or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. No. 2 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium

citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with the SSC buffer at 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The receptor of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the receptor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the receptor protein of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the receptor protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The cell lysate can be further purified by conventionally utilized chromatography procedures (e.g., gel filtration in an appropriately sized dextran or polyacrylamide column to separate the receptor protein). If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the receptor protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by tranfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from

those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG

(isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the receptor protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

One aspect of the present invention involves enhancing a plant's receptivity to treatment with a hypersensitive response elicitor by providing a transgenic plant or transgenic plant seed, transformed with a nucleic acid molecule encoding a receptor protein for a hypersensitive response elicitor. It has been found that hypersensitive response elicitors are useful in imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance in a variety of plants. In view of the receptor of the present invention's interaction with such elicitors, it is expected that these beneficial effects would be enhanced by carrying out such elicitor treatments with plants transformed with the receptor encoding gene of the present invention.

Transgenic plants containing a gene encoding a receptor in accordance with the present invention can be prepared according to techniques well known in the art.

A vector containing the receptor encoding gene described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes

allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing

transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds.

These elicitor treatment methods can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed transformed with a receptor gene in accordance with the present invention under conditions effective for the elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart resistance to stress.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance in the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant

transformed with both a DNA molecule encoding a receptor in accordance with the present invention and with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. The plant is grown under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and a DNA molecule encoding a receptor can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress.

The embodiment where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The hypersensitive response elicitor treatment can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of hypersensitive response elicitors in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia,* to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia,* to the following bacteria can also be imparted to plants *Pseudomonas solancearum*; *Pseudomonas syringae* pv. *tabaci*; and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia,* to the following fungi: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, there is significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

The use of hypersensitive response elicitors for insect control encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by

feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

Elicitor treatment is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

Hypersensitive response elicitor treatment is also useful in imparting resistance to plants against environmental stress. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients).

The application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or

injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with a hypersensitive response elicitor, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated with an elicitor, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or to impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative technique involving the use of transgenic plants and transgenic seeds encoding a hypersensitive response elicitor encoding gene, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art as described above.

In another embodiment, the present invention relates to a DNA construct which is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors. An example of such a construct would be an antisense DNA molecule of the DNA

molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3. Alternatively, the DNA construct can have a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3 (or a portion thereof) and its complementary strand and is used to generate a single transcript with an inverted repeat (i.e. a double-stranded) RNA. This transcript as well as the above-discussed antisense nucleic acid molecule can be used to induce silencing of a nucleic acid molecule encoding a receptor for a hypersensitive response elicitor.

Sensing the hypersensitive response elicitor by the receptor is the very first step of the signal transduction pathway in plants which eventually leads to disease resistance, growth enhancement, insect control, and stress resistance. Silencing the receptor provides a powerful tool to find and study the downstream components of this pathway. Additionally, the receptor could be a negative regulator of such plant signal transduction pathway. Silencing of the receptor will impart to plants the ability to resist disease and stress, control insects, and enhance growth without hypersensitive response elicitor treatment.

EXAMPLES

Example 1 - Materials and Methods

The laboratory technique used in the following example is straight forward. All DNA manipulations described here followed conventional protocols (Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory (1989); Ausubel, et al., "Current Protocols in Molecular Biology," John Wiley (1987), which are hereby incorporated by reference). The plasmids and microorganisms described herein, used for making the present invention, were obtained from commercial sources, or from the authors of previous publications. Sequences were analyzed with Clone Manager 5 (Scientific & Educational Software, Durham, North Carolina).

Yeast strain L40 was grown in YPD or in different minimal synthetic dropout selection media at 30°C. *E.coli* strains DH5α and HB101 were grown in LB at 37°C.

The yeast Two-Hybrid system is based on the fact that many eukaryotic transcription factors are composed of a physically separable, functionally independent DNA-binding domain (DNA-BD) and an activation domain (AD). Both the DNA-BD and the AD are required to activate a gene. When physically separated by recombinant DNA technology and expressed in the same host cell, the DNA-BD and the AD do not interact directly with each other and, thus, cannot activate the responsive gene (Ma, et al., "Converting a Eukaryotic Transcriptional Inhibitor into an Activator," Cell 55:443 (1988) and Brent, et al., "A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor," Cell 43:729 (1985), which are hereby incorporated by reference). But if the DNA-BD and the AD are brought into close physical proximity in the promoter region, the transcriptional activation function will be restored. Therefore, the yeast *Saccharomyces cerevisiae* and the Two-Hybrid system have become essential genetic tools for studying the macromolecular interactions.

In the Two-Hybrid system utilized here, the DNA-BD, encoded in the bait vector pVJL11 (Jullien-Flores, V., "Bridging Ral GTPase to Rho Pathways. RLIP76, a Ral Effector with CDC42/Rac GTPase-activating Protein Activity," J. Biol. Chem. 27:22473 (1995), which is hereby incorporated by reference), is the prokaryotic LexA protein, and the activation domain, encoded in the prey vector pGAD 10 or pGAD GH (Clontech; Hannon, GJ., "Isolation of the Rb-related p130 Through its Interaction with CDK2 and Cyclins," Genes Dev. 7:2378 (1993), which is hereby incorporated by reference) is derived from the yeast GAL4 protein. pVJL11 also has a *TRP1* marker, and the pGAD a *LEU2* marker. An interaction between the bait protein (fused to the DNA-BD) and a library-encoded protein (fused to the AD) creates a novel transcriptional activator with binding affinity for LexA operators. The yeast host L40 {MATa his3D200 trp1-901 leu2-3, 112 ade2 LYS2::$(lexAop)_4$-HIS3 URA3::$(lexAop)_8$-lacZ} harbors two reporter genes, *lacZ* and *HIS3*, which contain upstream LexA binding site. The *HIS3* nutritional reporter provides a sensitive growth selection that can identify a single positive transformant out of several million candidate clones. The expression of the reporter genes indicates interaction between a candidate protein and the bait protein. See Figure 1.

Erwinia amylovora harpin was used as the bait protein to screen the *Arabidopsis thaliana* MATCHMAKER cDNA library cloned in the pGAD 10 vector (Clontech Laboratories, Inc., Palo Alto, California). One cDNA library encoded protein was identified as a strong harpin interacting protein and, thus, a putative harpin receptor. The present invention reports the nucleic acid sequence and the deduced amino acid sequence of this cDNA.

Example 2

HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11. PCR was carried out using the 1.3 Kb harpin fragment (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992), which is hereby incorporated by reference) as a template to amplify the harpin encoding region. A Bam HI site was added to the 5' end of the coding sequence, and a Sal I site to the 3' end. A Bam HI and Sal I digested PCR fragment was ligated with the bait vector pVJL11 digested with the same restriction enzymes. pVJL11 has a *TRP1* marker to be selected in yeast and an Amp resistance marker to be selected in *E. coli*. The plasmid DNA was amplified in *E. coli* strain DH5α. When tested in the Two-Hybrid system with empty prey vector pGAD GH and several unrelated proteins, HrpN didn't show auto-activation or nonspecific interaction with unrelated proteins, as shown in Figure 2.

Example 3

HrpN-pVJL 11 was transformed into yeast strain L40 by a lithium acetate (LiAc)-mediated method (Ito et al., "Transformation of Intact Yeast Cells Treated with Alkali Cations," J. Bacteriol. 153:163 (1983) and Vojtek et al., "Mammalian Ras Interacts Directly with the Serine/Threonine Kinase Raf.," Cell 74:205 (1993), which are hereby incorporated by reference). The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for harpin interacting proteins. Approximately 6.8 million primary library transformants were plated onto plates lacking histidine, leucine, and tryptophan. A total of 148 colonies grew on the histidine dropout plates, 55 of which

stained positive when tested for expression of β-galactosidase. After three rounds of selection on synthetic minimal (SD) media plates lacking leucine, tryptophan, and histidine, and confirming by the expression of the second reporter gene *lacZ* using a β-galactosidase assay, 47 colonies seemed to be strong interacting candidates.

Example 4

Plasmid DNA was extracted from the 47 independent yeast colonies and shuttled into *E.coli* strain HB101, which carries the *leuB* mutation. Therefore, the prey plasmid (cDNA-pGAD 10) was selected for on minimal nutrient plates since pGAD 10 bears the *LEU2* marker.

The 47 independently rescued prey plasmids purified from *E. coli* were re-tested in the yeast two-hybrid system with harpin as bait. They were also tested against unrelated proteins. 25 turned out to be interacting candidates, 20 of which were strong specific interacting candidates. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The sequence reactions were performed using the PE Prism BigDye™ dye terminator reaction kit. The sequencing gel was run in Thatagen (Bothell, WA)

One of the eight plasmids, which had the longest cDNA insert of 1kb, was used for further analysis. When co-transformed into yeast strain L40, it was shown to be negative with empty bait and unrelated proteins in the Two-Hybrid system, indicating the specificity of the interaction between harpin and this receptor candidate. See Figure 3.

Example 5

The longest cDNA insert, HrBP1, was subcloned into the Bam HI and SalI sites of the bait vector pVJL 11. This construct didn't show auto-activation of the reporter genes, nor interaction with unrelated proteins in the yeast Two-Hybrid system. However, the expression of the reporter genes was activated when L40 was co-transformed with HrBP1-pVJL11 and HrpN-pGAD GH, indicating the specific interaction between HrBP1p (the protein encoded by HrBP1) and harpin. See Figure 4.

Example 6

Total RNA was extracted from two-week-old *Arabidopsis thaliana* using QIAGEN RNeasy plant mini kit (Qiagen, Inc., Valencia, California). Poly A^+ RNA was further purified from the total RNA with a QIAGEN Oligotex column (Qiagen, Inc., Valencia, California). A Northern blot was carried out using the translated region of HrBP1 as a probe. One single species with an apparent molecular weight of about 1.1 Kb was detected from both total RNA and Poly A^+ RNA. Therefore, the longest cDNA of HrBP1 from the yeast two-hybrid screen seems to be the full-length cDNA. The integrity of the 5' of cDNA was further confirmed by a primer extension assay.

As described, a yeast Two-Hybrid system was used to screen for harpin interacting proteins. HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11, which has a *TRP1* marker. The lexA harpin fusion protein is expressed from this construct in yeast. The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for hypersensitive response elicitor interacting proteins. 6.8 million independent colonies were screened, and HrBP1 was identified as a strong specific harpin interacting candidate. HrBP1 was mapped to *Arabidopsis thaliana* genomic DNA, chromosome 3, P1 clone MLM24 (Nakamura, "Structural Analysis of Arabidopsis thaliana chromosome 3," Direct submission to the DDBJ/EMBL/GenBank databases (1998), which is hereby incorporated by reference). Four exons and three introns were discovered (See Figure 5). Exon 4 includes a 130 bp non-translated 3' region. The in-frame open reading frame from the first methionine encodes a polypeptide (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is 30454.3 and pI is 5.72. There is no apparent hydrophobic trans-membrane domain in this polypeptide. SMART Simple Modular Architecture Research Tool (V3.1) predicted the first 18 amino acids as a signal sequence. The HrBP1-AD fusion prey was negative with empty bait and unrelated proteins in the yeast 2-H system, indicating the specificity of the interaction between harpin and this receptor candidate. When being put in the opposite orientation, i.e.

HrBP1p fused with the DNA-BD and harpin with the AD, they still specifically interacted with each other.

HrBP1 has no significant sequence similarity with sequences deposited in major sequence database accessible with the Blast search program. Therefore, HrBP1p is a novel protein.

Example 7

The HrBP1 cDNA was subcloned into the Nde I and Sal I sites of the vector pET-28a (Novagen, Madison, WI). HrBP1p was expressed from this vector in *E. coli* as a His-tagged protein and purified with Ni-NTA resion (QIAGEN Inc., Valencia, CA) according to the manual provided by the manufacturer. This recombinant protein increased harpin's ability to induce HR in tobacco plants. His-tag removed HrBP1 recombinant protein was used to generate anti-HrBP1 antibody to facilitate biochemical and functional studies of HrBP1. Preliminary localization study using anti-HrBP1 antibody in a Western blot showed that HrBP1p exists everywhere in *Arabidopsis*, including its leaves, stems, and roots.

Example 8

10μg of total RNA from 14 different plant species was separated on 1% agarose gel, and then transferred to Amersham Hybond NX membrane (Amersham Pharmacia Biotech, Piscataway, New Jersey). The RNA probe, which was complementary to bases 651-855 of HrBP1 coding region, was generated using Ambion Strip-EZ RNA kit (Ambion Inc., Houston, Texas). Membrane hybridization was done with Ambion ULTRAhyb (Ambion Inc., Houston, Texas), procedure according to manufacturer recommendation.

The sequence of the HrBP1 fragment used to generate the Northern probe (SEQ. ID. No. 9) is as follows:

```
gatcaagata acatttgaga aaacaactgt gaagacatcg ggaaacttgt cgcagattcc 60
tccgtttgat atcccgaggc ttcccgacag tttcagacca tcgtcaaacc ctggaactgg 120
ggatttcgaa gttacctatg ttgatgatac catgcgcata actcgcgggg acagaggtga 180
acttagggta ttcgtcattg cttaa                                      205
```

This Northern blot picked up a band with similar size as HrBP1 in all the plant species tested, including tobacco, wheat, corn, citrus, cotton, grass, pansy, pepper, potato, tomato, soybean, sun flower, and lima bean, which indicated HrBP1-like genes exist universally. See Figure 6.

Example 9

HrBP1 homologue from rice, R6, was clone by yeast two-hybrid screening using harpin as bait. It not only interacted with full length harpin but also interacted with a harpin fragment that contains the second HR domain (see Figure 7). However, it is not a full-length cDNA; there is some sequence information missing from the 5' end. The partial sequence of HrBP1-like cDNA from rice encodes a peptide of 203 amino acids, R6-p, which starts at amino acid 84 of HrBP1p. They are 74.4% identical and 87.2% positive at the protein level, they are 65% identical at the DNA level.

The following shows the sequence alignment of HrBP1 (SEQ. ID. No. 1 starting at amino acid 84) and R6 (SEQ. ID. No. 4) at the protein level:

At protein level: Identities = 151/203 (74.4%), Positives = 177/203 (87.2%), Gaps = 2/203 (0%)

```
R6-p   : 1   VAALKVKLLSAVSGLNRGLAGSQEDLDRADAAARELEAAAGGGPVDLERDVDKLQGRWRL
             +A LK+KLLS VSGLNRGL  S +DL+RA+ AA+ELE A  GGPVDL  D+DKLQG+WRL
HrBP1p: 84   IALLKLKLLSVVSGLNRGLVASVDDLERAEVAAKELETA--GGPVDLTDDLDKLQGKWRL

R6-p   : 61  VYSSAFSSRTLGGSRPGPPTGRLLPITLGQVFQRIDVVSKDFDNIVDVELGAPWPLPPVE
             +YSSAFSSR+LGGSRPG PTGRL+P+TLGQVFQRIDV SKDFDNI +VELGAPWP PP+E
HrBP1p: 142 LYSSAFSSRSLGGSRPGLPTGRLIPVTLGQVFQRIDVFSKDFDNIAEVELGAPWPFPPLE

R6-p   : 121 LTATLAHKFEIIGTSSIKITFDKTTVKTKGNLSQLPPLEVPRIPDNLRPPSNTGSGEFEV
              TATLAHKFE++GT  IKITF+KTTVKT GNLSQ+PP ++PR+PD+ RP SN G+G+FEV
HrBP1p: 202 ATATLAHKFELLGTCKIKITFEKTTVKTSGNLSQIPPFDIPRLPDSFRPSSNPGTGDFEV

R6-p   : 181 TYLDGDTRITRGDRGELRVFVIS 203
             TY+D   RITRGDRGELRVFVI+
HrBP1p: 262 TYVDDTMRITRGDRGELRVFVIA 284
```

The sequence alignment, on a DNA level, of R6 (SEQ. ID. No. 5) and HrBP1 (SEQ. ID. No. 2) starting at nucleotide 265 (i.e. nucleotide 249 of the open reading frame))

```
At DNA level: Identities = 397/610 (65%)   (dots indicate identical bases)

R6      1    cgtggctgcgctcaaagtcaagcttctgagcgcggtgtccgggctgaaccgcggcctcgc
HrBP1   249  aa.t..atta.....c....at.a..t..t.ta..t..g..at.a...a.a..a..t.t
```

```
R6      61   ggggagccaggaggatcttgaccgcgccgacgcggcggcgcgggagctcgaggcggcggc
HrBP1   309  ..c...tgtt..t...t.a..aa.a..t..a.t...t..taaa..a..t..aa.t..---

R6      121  gggtggcggccccgtcgacctggagagggacgtggacaagctgcaggggcggtggaggct
HrBP1   366  ---...g..a..g..t..tt.aaccgat..tc.t..t.....t..a...aaa........

R6      181  ggtgtacagcagcgcgttctcgtcgcggacgctcggcggcagccgccccggcccgcccac
HrBP1   423  .t....t..t..t........t..t...t.tt.a..t..t.....t..t..t.ta..t..

R6      241  cggccgcctcctccccatcaccctcgggcaggtgtttcagaggatcgatgttgtcagcaa
HrBP1   483  t..a..tt.ga....tg.t..t..t..c...........ac....t.....gt.t.....

R6      301  ggacttcgacaacatcgtcgatgtcgagctcggcgcgccatggccgctgccgccggtgga
HrBP1   543  a..t..t..t.....a.ca..g..g..at.a..a..c..t.....at.t.....at.a..

R6      361  gctgacggcgaccctggctcacaagtttgagatcatcggcacctcgagcataaagatcac
HrBP1   603  agcc..t.....at....a...........ac..t.a.....t.gc.ag..c.....a..

R6      421  attcgacaagacgacggtgaagacgaaggggaacctgtcccagctgccgccgctggaggt
HrBP1   663  ...t..g..a..a..t........atc...a...t....g...a.t..t...t.t..ta.

R6      481  ccctcgcatcccggacaacctccggccgccgtccaacaccggcagcggcgagttcgaggt
HrBP1   723  ...ga.gc.t..c....gtt..a.a..at....a...c.t..a.ct..g..t.....a..

R6      541  gacctacctcgacggcgacacccgcatcacccgcggggacagaggggagctcagggtgtt
HrBP1   783  t.....tg.t..t.atac..tg.....a..t..............t..a..t.....a..

R6      601  cgtcatctcg
HrBP1   843  ......tg.t
```

Example 10

Arabidopsis thaliana Columbia plants were grown in autoclaved potting mix in a controlled environment room at a day and night temperature of 23-20°C and a photoperiod of 14 h light.

A transgenic approach was used for functional analysis of HrBP1. Anti-sense HrBP1, which is complementary to SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212, and is under the control of NOS promoter. *Arabidopsis thaliana* plants were transformed with this construct via an Agrobacteria mediated method. The *Agrobacterium tumefaciens* strain used was GV3101 (C58C1 Rifr) pMP90 (Gmr). These antisense lines were designated "as" lines.

Arabidopsis plants were also transformed with a construct, which has an inverted repeat with a sense strand of HrBP1 coding region bases 4-650 (i.e. bases 20-666 of SEC. ID. No. 2) and the complementary sequence of bases 20-516 of HrBP1 cDNA (i.e. SEQ. ID. No. 2). This construct generated a double-stranded mRNA in transformed plants. These transgenic lines were designated "d" lines.

Figure 8 shows the constructs used to transform *Arabidopsis*.

Both antisense and double-stranded approaches were to silence the expression of HrBP1. The double stranded RNA method was found to be more efficient in silencing the HrBP1 gene. Some transgenic *Arabidopsis* lines showed spontaneous HR-mimic lesion. The most severe line was developmentally retarded, looked very sick, and did not produce seeds.

Example 11

Plants were grown in autoclaved potting mix in a controlled environment room with a day and night temperature of 23-20°C and a photoperiod of 14 h light. 25-day-old plants were inoculated with *Pseudomonas syringae* p.v. tomato DC3000 by dipping the above soil parts of the plants in 10^8 cells ml^{-1} bacteria suspension for 10 second. Seven days after DC3000 inoculation, leaf disks were harvested with cork borer. Bacteria were extracted from leaf disk in 10mM $MgCl_2$ and plated on King's B agar containing 100 μg/ml rifampicin. Plates were incubated at 28°C for 2 days (Figure 9B) and colonies counted. In Figure 9A, wild type *Arabidopsis* plants had significantly more disease development than transgenic plants. Bacteria counting (Figure 9C) showed that transgenic plants had at least one magnitude less of DC3000 growing inside the leaves. HrBP1 seemed like a negative regulator of plant defense signal transduction pathway in *Arabidopsis*. Its silencing imparted plants with the ability to resist *Pseudomonas syringae* p.v. tomato DC3000.

Example 12

HrBP1 coding region, bases 17-871 of SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212 which is under the control of the NOS promoter (see Figure 10). Tobacco plants were transformed with this construct via an *Agrobacteria* mediated method. The *Agrobacterium tumefaciens* strain used was LBA4404.

Example 13

HrBP1 was over-expressed in tobacco plants under the control of an NOS promoter. Figure 10 shows the construct used for tobacco transformation. Three high expression lines were chosen for further studies in the T2 generation.

When infiltrated with purified harpin, the transgenic lines developed HR much faster than wild type plants, which is consistent with previous experiment in which His-tagged HrBP1 increased tobacco's sensitivity to harpin protein. The HrBP1 over-expressing lines were about 20-30% taller than wild type Xanthi NN plants (see Figure 11).

Example 14

61-day-old wild type and HrBP1 over-expressing Xanthi NN tobacco plants were inoculated with tobacco mosaic virus by rubbing TMV with diatomaceous earth on the upper surface of leaves. Lesions appeared 2 days after manual inoculation. The picture in Figure 12A was taken 3 days after inoculation. The diameter of disease spots was measured. On average, the diameter of lesions on transgenic plant leaves were 33.4% less than that on wild type plants (Figure 12B). Therefore, the surface area of lesions on transgenic plant leaves was about 44.3% of those of the wild type plants. HrBP1 seemed to be a positive regulator of the plant signal transduction pathway for growth and disease resistance in tobacco.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. An isolated protein which serves as a receptor in plants for plant pathogen hypersensitive response elicitors.

2. A protein according to claim 1, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, and *Clavibacter*.

3. A protein according to claim 2, wherein the plant pathogen is an *Erwinia* pathogen.

4. A protein according to claim 3, wherein the plant pathogen is *Erwinia amylovora*.

5. A protein according to claim 1, wherein the protein is from a monocot.

6. A protein according to claim 5, wherein the protein is from rice.

7. A protein according to claim 1, wherein the protein has a partial amino acid sequence of SEQ. ID. No. 4.

8. A protein according to claim 1, wherein the protein is from a dicot.

9. A protein according to claim 8, wherein the protein is from *Arabidopsis thaliana*.

10. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

11. A protein according to claim 1, wherein the protein is recombinant.

12. An isolated nucleic acid molecule encoding a protein according to claim 1.

13. A nucleic acid molecule according to claim 12, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, and *Clavibacter*.

14. A nucleic acid molecule according to claim 13, wherein the plant pathogen is an *Erwinia* pathogen.

15. A nucleic acid molecule according to claim 14, wherein the plant pathogen is *Erwinia amylovora*.

16. A nucleic acid molecule according to claim 12, wherein the protein is from a monocot.

17. A nucleic acid molecule according to claim 16, wherein the protein is from rice.

18. A nucleic acid molecule according to claim 12, wherein the protein has a partial amino acid sequence of SEQ. ID. No. 4.

19. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C.

20. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 5.

21. A nucleic acid molecule according to claim 12, wherein the protein is from a dicot.

22. A nucleic acid molecule according to claim 21, wherein the protein is from *Arabidopsis thaliana*.

23. A nucleic acid molecule according to claim 12, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

24. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

25. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 2.

26. A nucleic acid according to claim 12, wherein the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

27. A nucleic acid according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 3.

28. An antisense nucleic acid molecule to the nucleic acid according to claim 12.

29. An expression vector containing a nucleic acid molecule according to claim 12 which is heterologous to the expression vector.

30. An expression vector according to claim 29, wherein the nucleic acid molecule is positioned in the expression vector in sense orientation and correct reading frame.

31. An expression vector according to claim 29, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

32. An expression vector containing a nucleic acid molecule according to claim 28 which is heterologous to the expression vector.

33. A transgenic host cell transformed with the nucleic acid molecule according to claim 12.

34. A host cell transformed according to claim 33, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

35. A host cell according to claim 33, wherein the DNA molecule is transformed with an expression system.

36. A host cell according to claim 33, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

37. A host cell transformed with a nucleic acid molecule according to claim 28.

38. A transgenic plant transformed with the DNA molecule of claim 12.

39. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

40. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

41. A transgenic plant according to claim 38, wherein the plant is a monocot.

42. A transgenic plant according to claim 38, wherein the plant is from a dicot.

43. A transgenic plant according to claim 38, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

44. A transgenic plant transformed with a nucleic acid molecule according to claim 28.

45. A transgenic plant seed transformed with the DNA molecule of claim 12.

46. A transgenic plant seed according to claim 45, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber,

apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

47. A transgenic plant seed according to claim 45, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

48. A transgenic plant seed according to claim 45, wherein the plant is a monocot.

49. A transgenic plant seed according to claim 45, wherein the plant is a dicot.

50. A transgenic plant seed according to claim 45, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0:9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

51. A transgenic plant seed transformed with a nucleic acid molecule according to claim 28.

52. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a protein according to claim 1 and a candidate agent;

evaluating the reaction mixture for binding between the protein and the candidate agent; and

identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

53. A method according to claim 52, wherein the protein is from a monocot.

54. A method according to claim 53, wherein the protein is from rice.

55. A method according to claim 52, wherein the protein has an amino acid sequence comprises SEQ. ID. No. 4.

56. A method according to claim 52, wherein the protein is from a dicot.

57. A method according to claim 56, wherein the protein is from *Arabidopsis thaliana*.

58. A method according to claim 52, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

59. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a host cell transformed with a nucleic acid molecule according to claim 12 and a candidate agent;

evaluating the reaction mixture for binding between protein produced by the host cell and the candidate agent; and

identifying candidate compounds which bind to the protein produced by the host cell in the reaction mixture as plant cell targeting agents.

60. A method according to claim 59, wherein the protein is from a monocot.

61. A method according to claim 60, wherein the protein is from rice.

62. A method according ~~to claim 59, wherein the protein is from a~~ dicot.

63. A method according to claim 62, wherein the protein is from *Arabidopsis thaliana*.

64. A method according to claim 59, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

65. A method of enhancing plant receptivity to treatment with hypersensitive response elicitors comprising:

providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

66. A method according to claim 65, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

67. A method according to claim 65, wherein a transgenic plant is provided.

68. A method according to claim 65, wherein a transgenic plant seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

69. A method according to claim 65, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

70. A method according to claim 65, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

71. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting disease resistance.

72. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for enhancing plant growth.

73. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for controlling insects.

74. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting stress tolerance.

75. A method according to claim 65, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects the hypersensitive response elicitor treatment.

76. A method according to claim 65, wherein the hypersensitive response elicitor treatment comprises:

applying a hypersensitive response elicitor to the plant or plant seed.

77. A method according to claim 76, wherein the hypersensitive response elicitor is applied in isolated form.

78. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:

providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule according to claim 12.

79. A method according to claim 78, wherein the protein is from a monocot.

80. A method according to claim 79, wherein the protein is from rice.

81. A method according to claim 78, wherein the protein is from a dicot.

82. A method according to claim 81, wherein the protein is from *Arabidopsis thaliana*.

83. A method according to claim 78, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

84. A method according to claim 78, wherein a transgenic plant is provided.

85. A method according to claim 78, wherein a transgenic plant seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

86. A method according to claim 78, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, ~~turnip, radish, spinach, onion,~~ garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

87. A method according to claim 78, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

88. A method according to claim 78, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects a hypersensitive response elicitor treatment.

89. A method according to claim 78 further comprising:

applying a hypersensitive response elicitor to the plant or plant seed.

90. A method according to claim 89, wherein the hypersensitive response elicitor is applied in isolated form.

91. A method according to claim 78, wherein disease resistance is imparted to plants.

92. A method according to claim 78, wherein enhanced growth is imparted to plants.

93. A method according to claim 78, wherein insect control is imparted to plants.

94. A method according to claim 78, wherein stress resistance is imparted to plants.

95. A method according to claim 78, wherein the DNA construct is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors.

96. A method according to claim 78, wherein the DNA construct is transcribable to a first nucleic acid encoding a receptor in plants for plant pathogen hypersensitive response elicitors coupled to a second nucleic acid encoding the inverted complement of the first nucleic acid.

97. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:

providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

98. A method according to claim 97, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid

sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

99. A method according to claim 97, wherein a transgenic plant is provided.

100. A method according to claim 97, ~~wherein a transgenic plant~~ seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

101. A method according to claim 97, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

102. A method according to claim 97, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

103. A method according to claim 97, wherein disease resistance is imparted.

104. A method according to claim 97, wherein plant growth is enhanced.

105. A method according to claim 97, wherein insects are controlled.

106. A method according to claim 97, wherein stress tolerance is imparted.

107. A method according to claim 97, wherein the protein is from a monocot.

108. A method according to claim 107, wherein the protein is from rice.

109. A method according to claim 97, wherein the protein is from a dicot.

110. A method according to claim 109, wherein the protein is from *Arabidopsis thaliana*.

SEQUENCE LISTING

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35 40 45

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50 55 60

Ala Phe Ser Ser Arg Thr Leu Gly Gly Ser Arg Pro Gly Pro Pro Thr
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Gly Arg Leu Leu Pro Ile Thr Leu Gly Gln Val Phe Gln Arg Ile Asp
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Val Val Ser Lys Asp Phe Asp Asn Ile Val Asp Val Glu Leu Gly Ala
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Pro Arg Ile Pro Asp Asn Leu Arg Pro Pro Ser Asn Thr Gly Ser Gly
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Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile
        35                  40                  45

Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys
    50                  55                  60

Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu
65                  70                  75                  80

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                85                  90                  95
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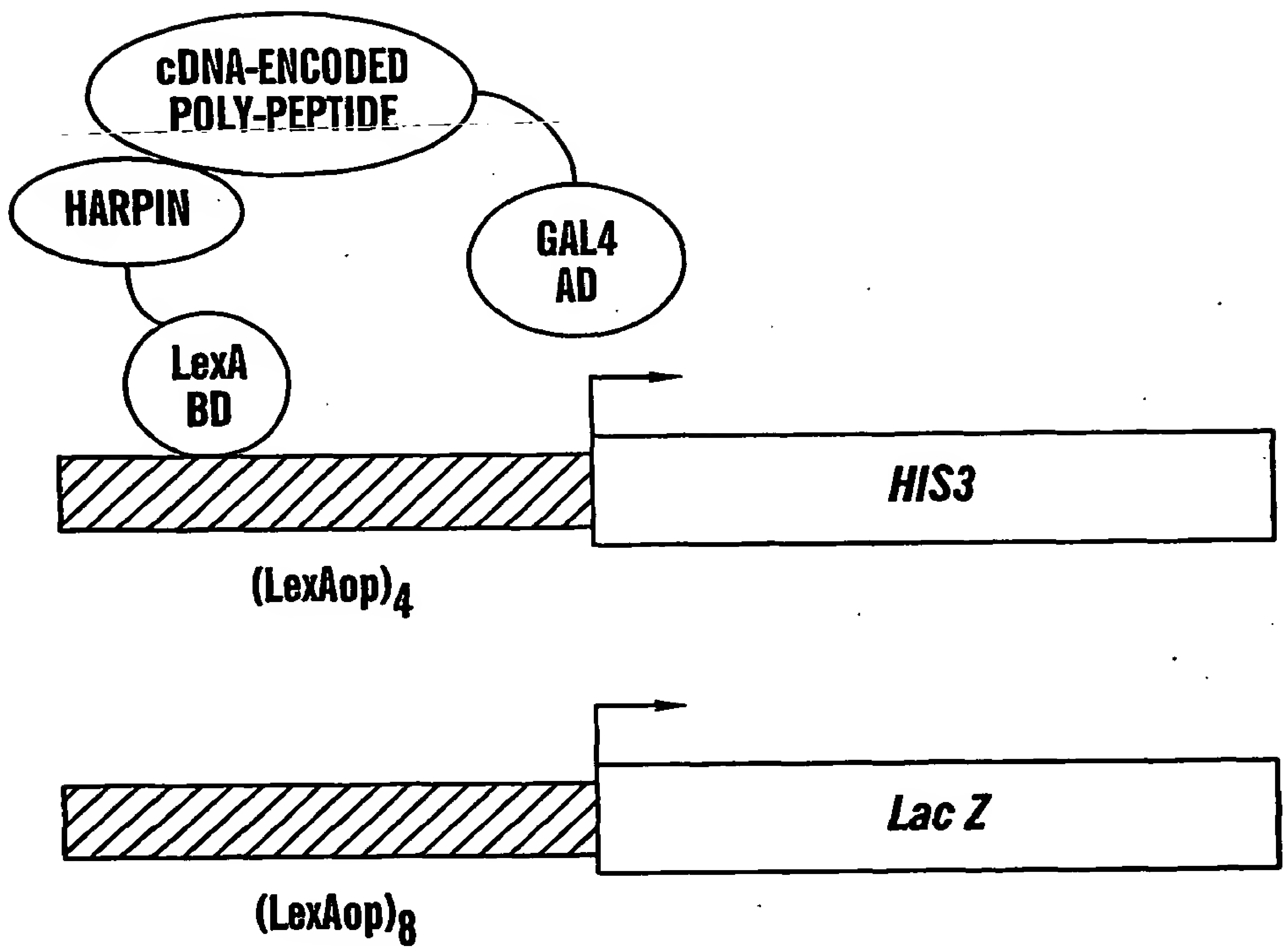
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HIS3
(LexAop)4
Lac Z
(LexAop)8

FIG. 1

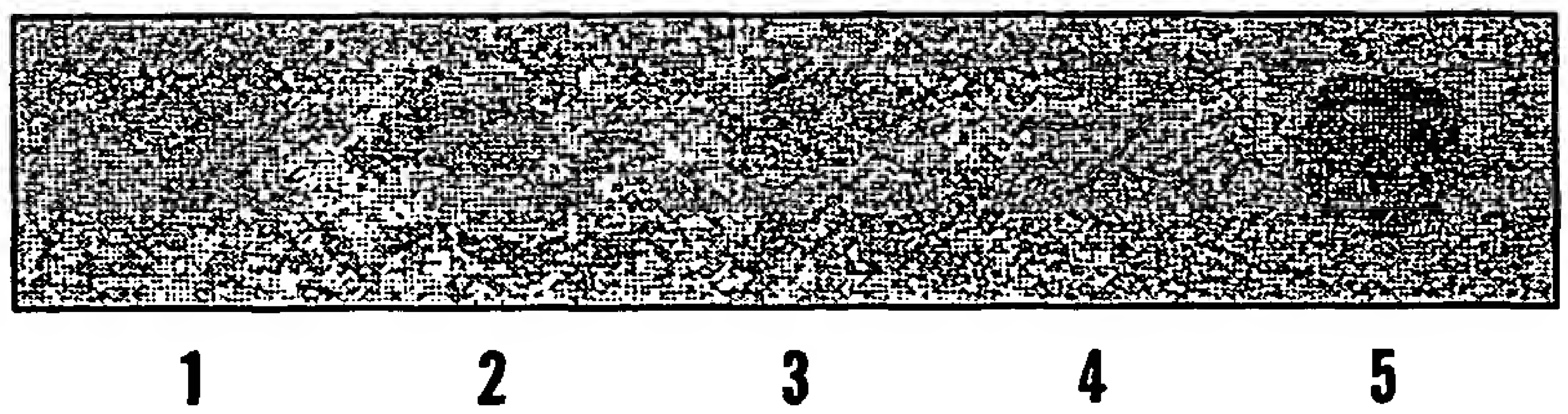
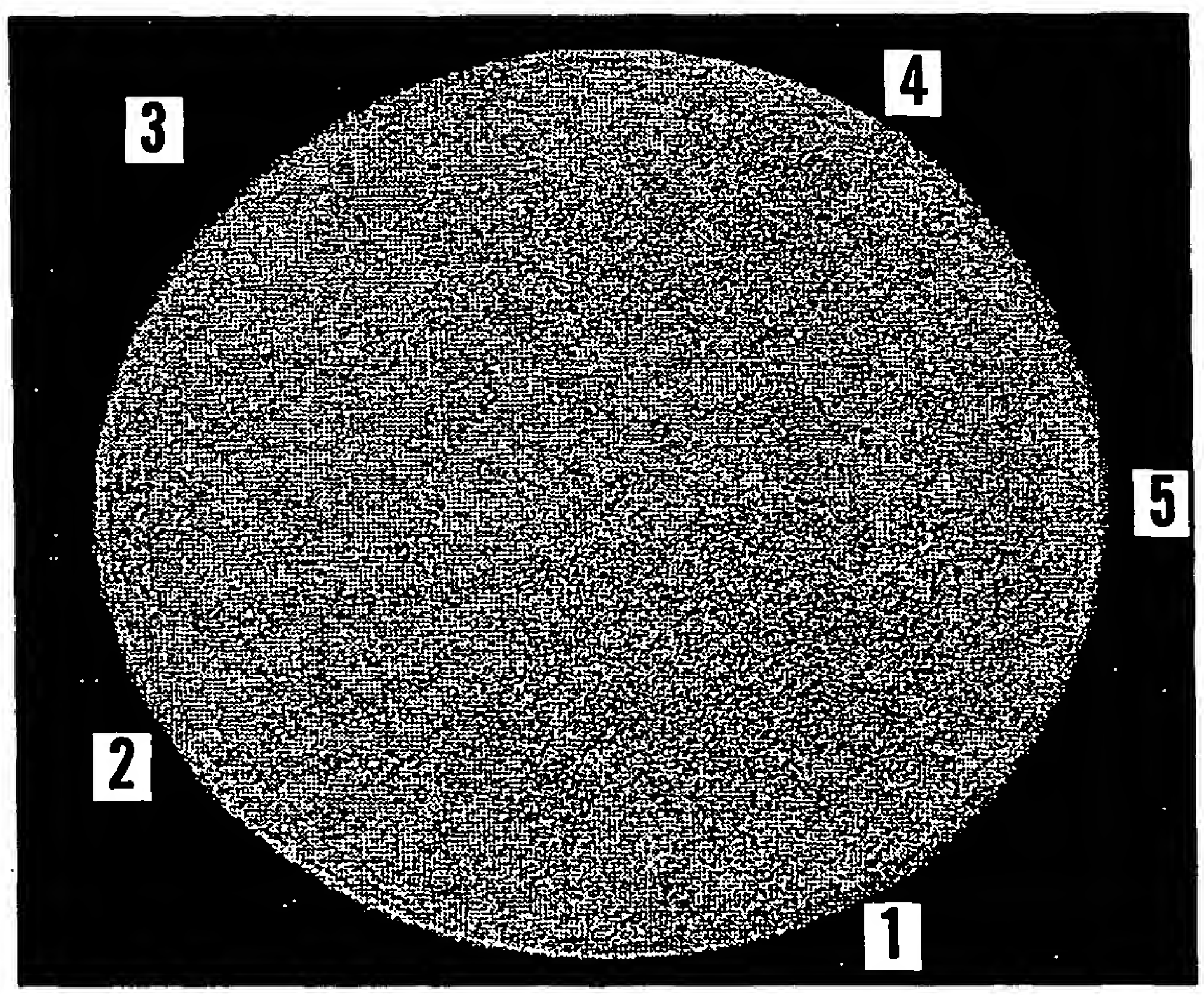


FIG. 2A



	BAIT	PREY
1	HARPIN	EMPTY VECTOR
2	HARPIN	UNRELATED PROTEIN
3	EMPTY VECTOR	HARPIN
4	UNRELATED PROTEIN	HARPIN
5	(POSITIVE CONTROL)	

FIG. 2B

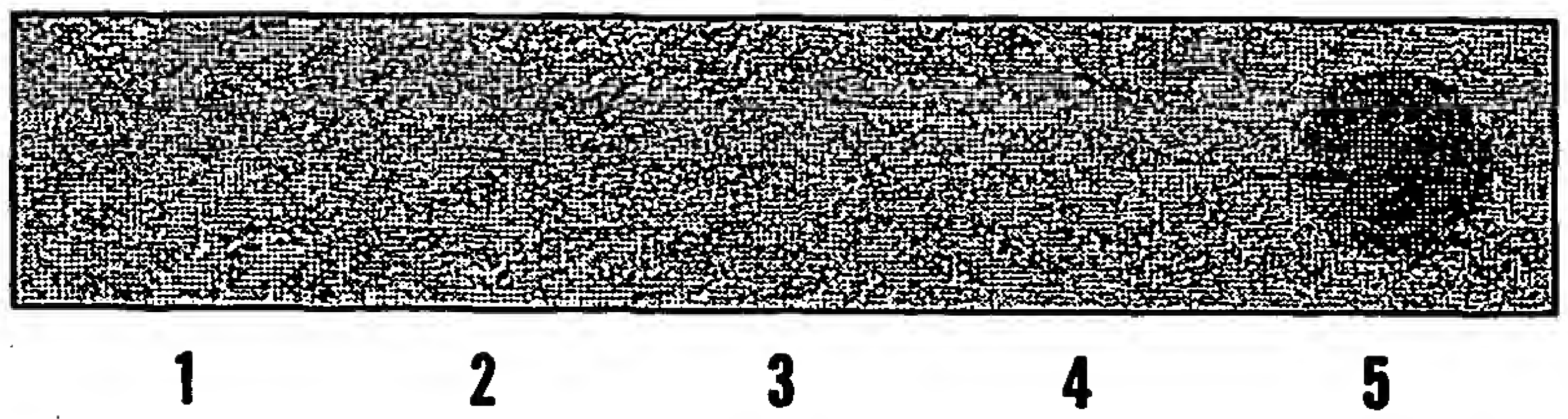
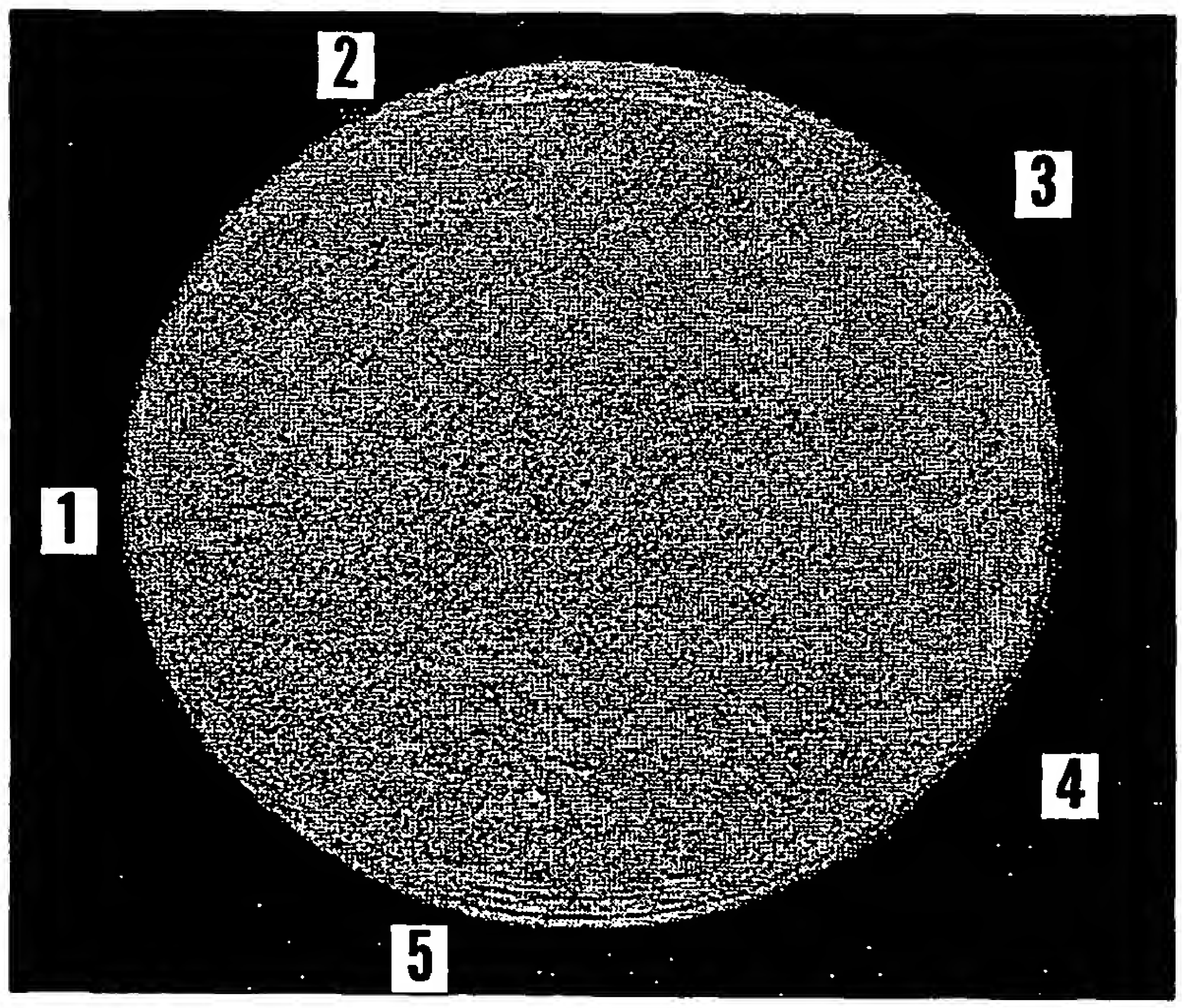


FIG. 3A



	BAIT	PREY
1	HARPIN	HrBP1
2	UNRELATED PROTEIN 1	HrBP1
3	UNRELATED PROTEIN 2	HrBP1
4	EMPTY VECTOR	HrBP1
5	(POSITIVE CONTROL)	

FIG. 3B

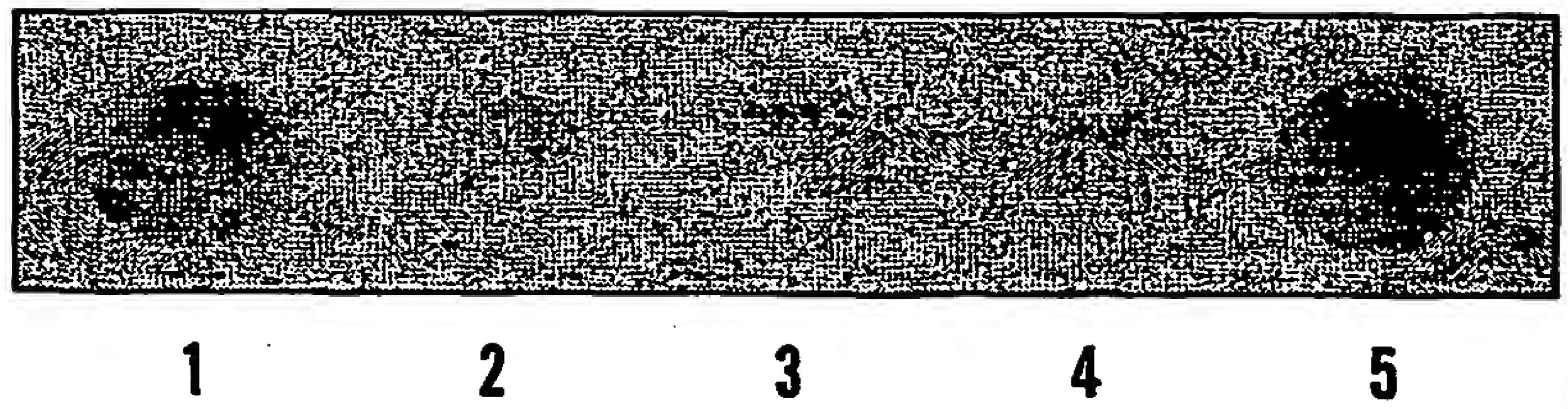
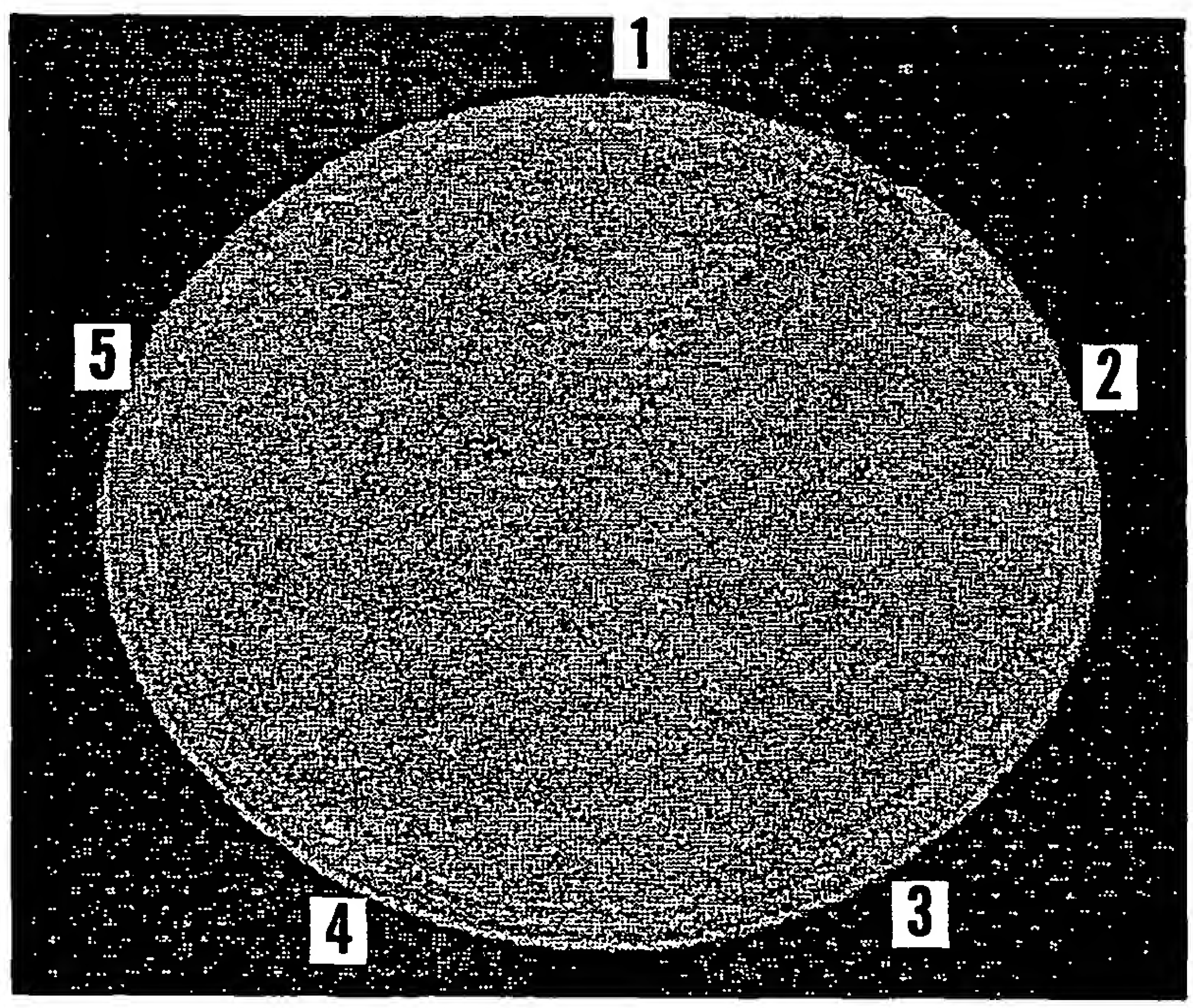
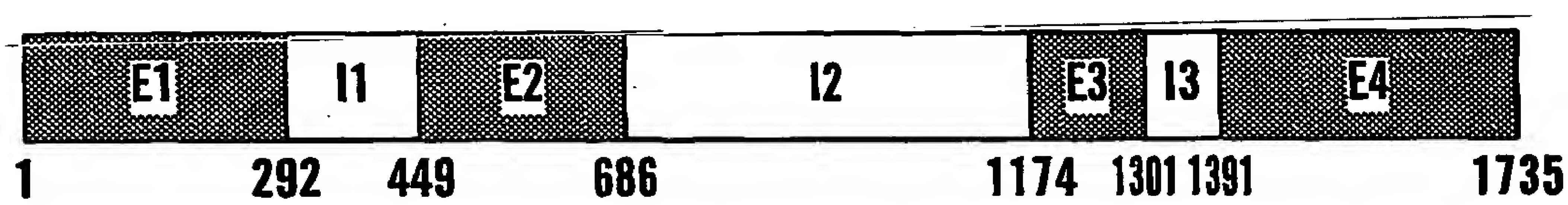


FIG. 4A



	BAIT	PREY
1	HrBP1	HARPIN
2	HrBP1	EMPTY VECTOR
3	HrBP1	UNRELATED PROTEIN 1
4	HrBP1	UNRELATED PROTEIN 2
5	(POSITIVE CONTROL)	

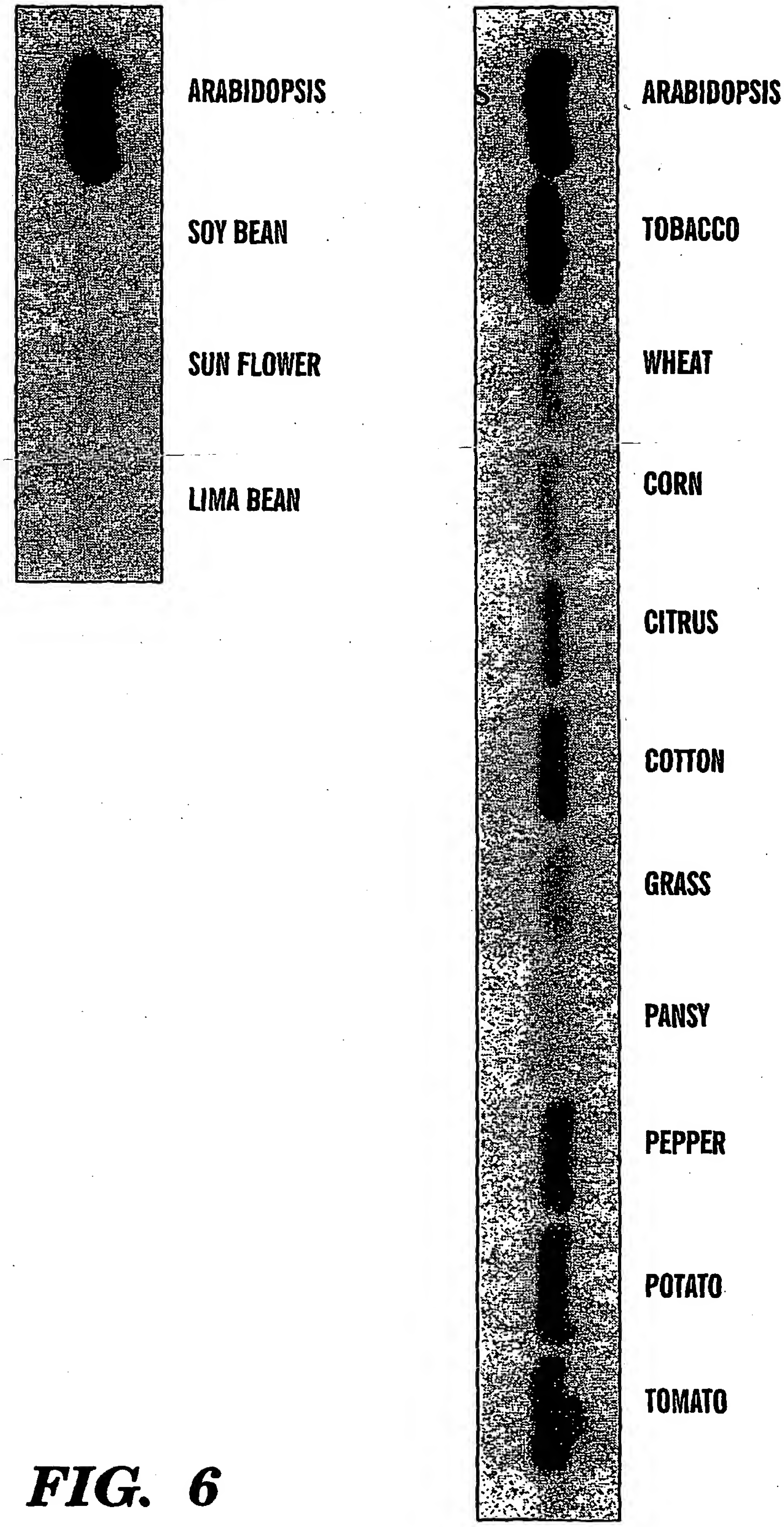
FIG 4B



GENE STRUCTURE OF HrBP1

E: EXON I: INTRON

FIG. 5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SUN FLOWER
LIMA BEAN
ARABIDOPSIS
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PANSY
PEPPER
POTATO
TOMATO

FIG. 6

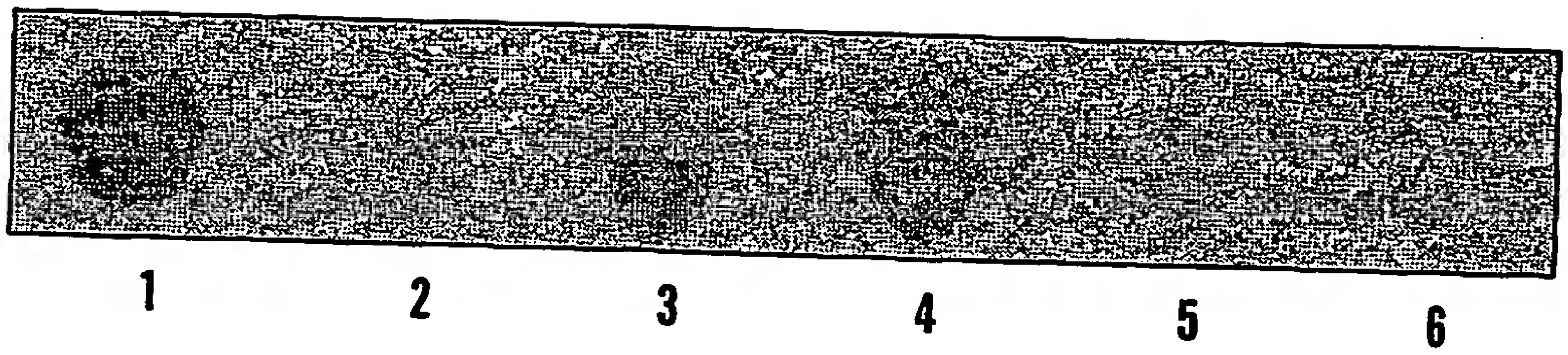
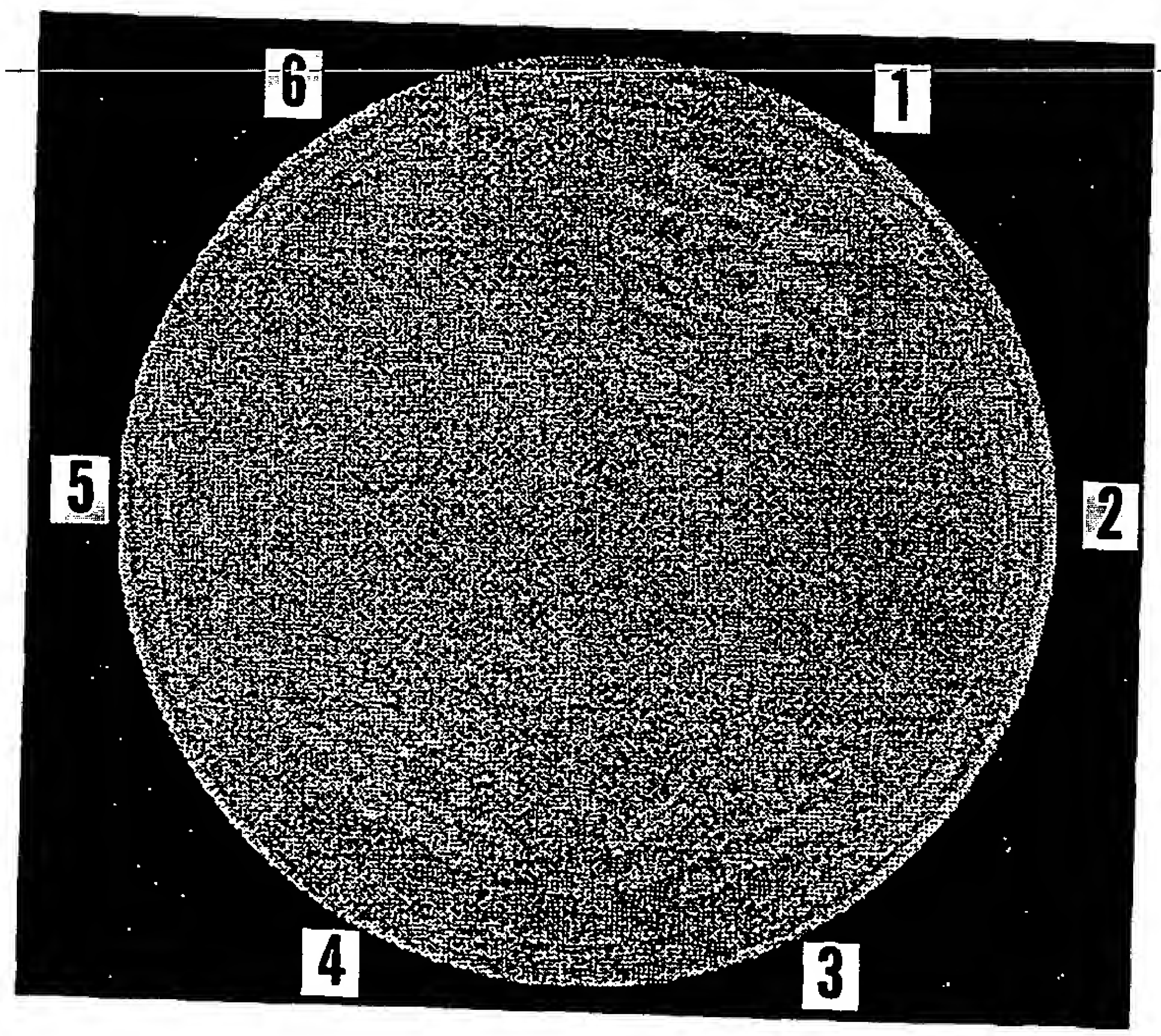
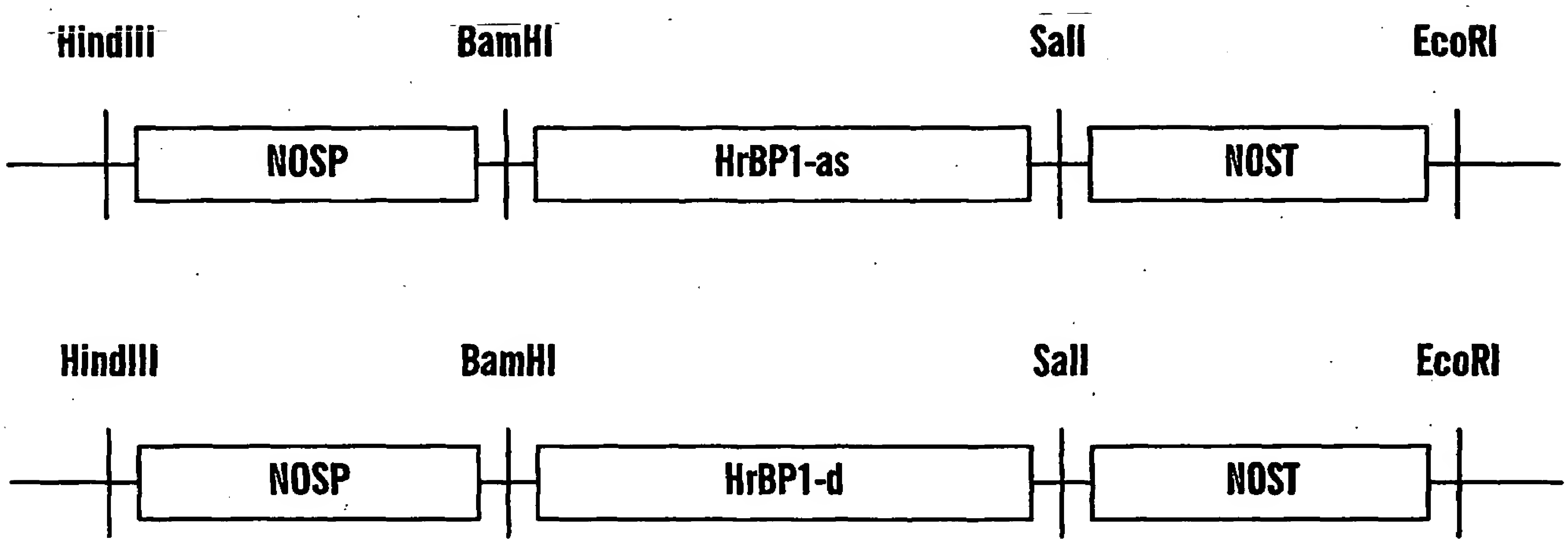


FIG. 7A



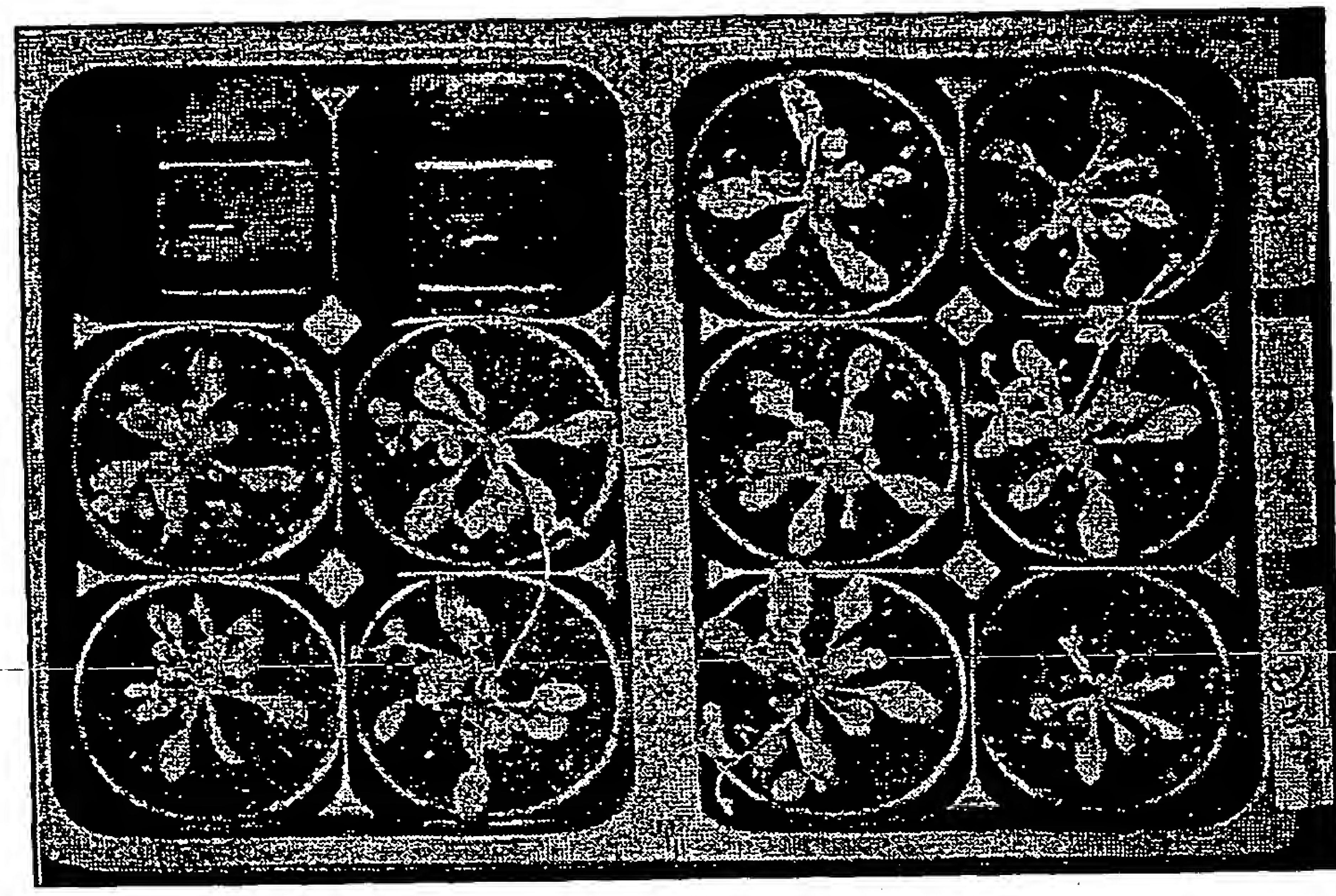
	BAIT	PREY
1	POSITIVE CONTROL	
2	pVJL11	R6
3	hrpN FULL LENGTH	R6
4	hrpN 137-180aa	R6
5	hrpN 210-403aa	R6
6	lexA-lamin	R6

FIG. 7B



HrBP1-d: HrBP1 CODING REGION BASES 4-650 SENSE STRAND + 4-500 ANTI-SENSE STRAND

FIG. 8



WILD TYPE

TRANSGENIC LINES

FIG. 9A

WILD TYPE

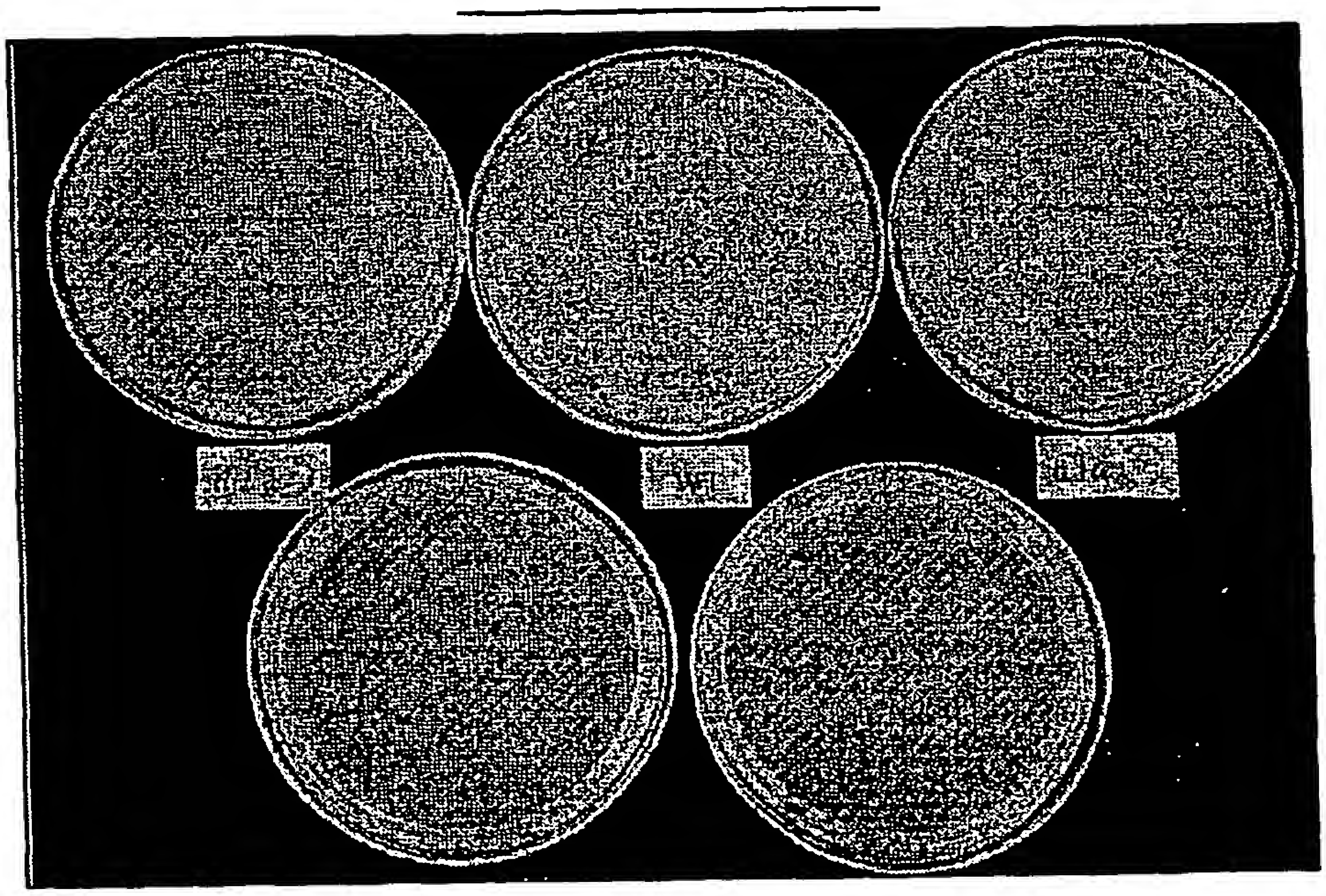


FIG. 9B

	cfu/cm^2
WILD TYPE	5.5×10^7
d2-2	2.0×10^5
d16-9	2.0×10^5
as14-7	2.1×10^6
as17-8	4.1×10^6

FIG 9C

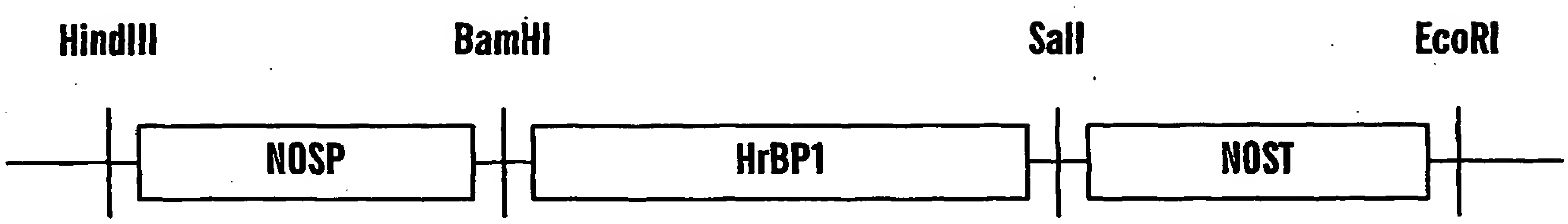


FIG. 10

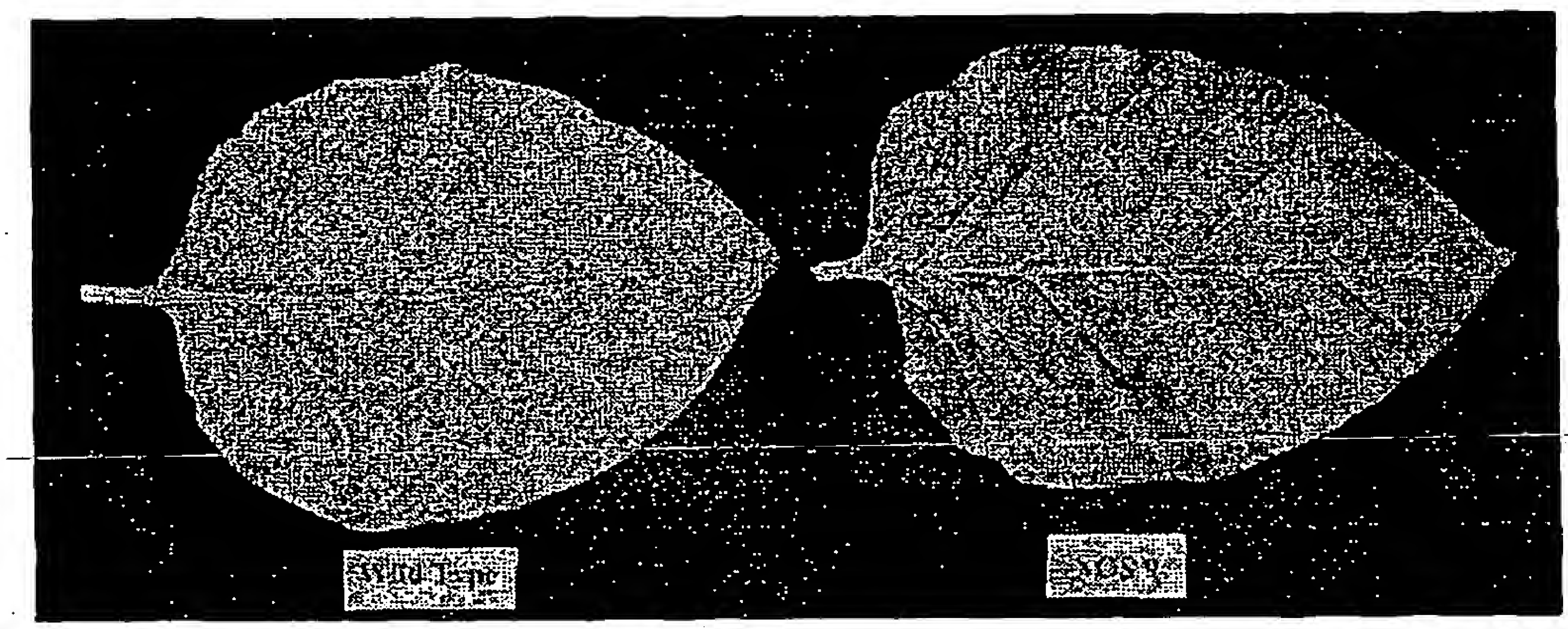
WILD TYPE HrPP1 OVER-EXPRESSING TOBACCO

FIG. 11A

HEIGHT OF T2 TOBACCO PLANTS AFTER 52 DAYS TRANSFERRING TO SOIL

WILD TYPE	49.5 ± 3.2 cm
LINE 1	59.5 ± 3.8 cm
LINE 2	61.7 ± 1.9 cm
LINE 3	64.3 ± 5.5 cm

FIG. 11B



WILD TYPE HrBP1 OVER-EXPRESSING LINE

FIG. 12A

	DIAMETER OF DISEASE SPOT
WILD TYPE	2.97 ± 0.49mm
LINE 1	1.97 ± 0.31mm
LINE 2	1.95 ± 0.37mm
LINE 3	2.01 ± 0.33mm

FIG. 12B

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CORRECTED VERSION*

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(74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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With international search report.

(88) Date of publication of the international search report: 6 July 2000 (06.07.00)

(54) Title: HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

(57) Abstract

The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SEP 28 2000

HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/103,050, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to active fragments of a hypersensitive response elicitor which fragments do not elicit a hypersensitive response.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora,*" Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* $\text{Harpin}_{\text{Ech}}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA^- Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240
```

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275 280 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290 295 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
325 330 335

Asn Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

```
CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG    60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC   120
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG   180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG   240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG   300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG   360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC   420
CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT   480
CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG   540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA   600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC   660
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT   720
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT   780
GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC   840
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TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA    900
TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC    960
CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC   1020
CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG   1080
CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT   1140
GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT   1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA   1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA   1320
TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA   1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG   1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA   1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC   1560
GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA   1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC   1680
TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA   1740
ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC   1800
GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC   1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG   1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG   1980
GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC   2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG   2100
GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T                       2141
```

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 23 as follows:

```
Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                   10                  15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30
```

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
50 55 60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
180 185 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
210 215 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
245 250 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
275 280 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
290 295 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305 310 315 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
325 330 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn

```
                    340                      345                      350
Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
        355                     360                     365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                     375                     380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                     390                     395                     400

Gly Ala Ala
```

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

```
AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA     60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT    120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG    180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG    240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG    300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA    360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA    420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC    480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC    540
CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG    600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC    660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG    720
CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC    780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG    840
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TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT 900

ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960

GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC 1020

CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC 1080

AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140

ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200

GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260

CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG 60

GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120

CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180

CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240

AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300

CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360

CAGGGCGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA 420

CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480

TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC 540

CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCATCC 600

ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG 660

GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720

AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780

GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA 840

GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC 900

CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC 960

AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020

AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC 1080

AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140

TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200

CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260

GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320

GCCAACCTGA AGGTGGCTGA ATGA 1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                   10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                  80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220
```

```
Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

```
ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC    60
CCTGTGGGGC ATGGTGTTGC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC   120
GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA   180
TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG   240
GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC   300
CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT   360
GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT   420
ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA   480
ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC   540
AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTC ACCGCCGCAC   600
ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA   660
ACGGCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA   720
CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC   780
GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC   840
CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT   900
GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG   960
GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC  1020
TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC  1080
CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC  1140
GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA  1200
AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAAGCCAA  1260
CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG  1320
CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG  1380
CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC  1440
GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC  1500
TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG  1560
ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC  1620
CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC  1680
GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTTGTGGCC  1740
GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG  1800
AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT  1860
TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG  1920
CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT  1980
```

GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG 2040
ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT 2100
GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC 2160
CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG 2220
AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT 2280
AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG 2340
GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC 2400
CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACTCTCAG CCGCGAAGGT 2460
ATCAGCGGCG AACTGAAAGA CATTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC 2520
CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC 2580
AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC 2640
CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC 2700
GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA 2760
CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG 2820
TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC 2880
AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG 2940
CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG 3000
AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCGT 3060
CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACTGGAAA CTCTGGATTT AGGCGAACAT 3120
GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC 3180
CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT 3240
AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT 3300
GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG 3360
AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG 3420
AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA 3480
TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG 3540
AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC 3600
GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC 3660
TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC 3720
TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC 3780
AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT 3840
ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC 3900

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AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC   3960
CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG   4020
AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA   4080
ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG   4140
CGTATCGGCG CTGCTGTGAG TGGCACCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT   4200
AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC   4260
CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG   4320
TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC   4380
GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAC   4440
CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG   4500
GCCAGCAATA ACCGCCCAAC CTTCCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT   4560
GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCGGGAT CTTCCCGGCA   4620
TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC   4680
AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC   4740
ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG   4800
TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA   4860
GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT   4920
CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC   4980
AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTCGAT   5040
GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA   5100
CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG   5160
GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT   5220
CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC   5280
GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG   5340
AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC   5400
CAGGATCAGA ACACCCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG   5460
CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA      5517
```

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

```
Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
1               5                   10                  15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
            20                  25                  30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
        35                  40                  45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
    50                  55                  60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65                  70                  75                  80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
                85                  90                  95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
            100                 105                 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
        115                 120                 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
    130                 135                 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145                 150                 155                 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
                165                 170                 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
            180                 185                 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
        195                 200                 205

Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
    210                 215                 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
225                 230                 235                 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
                245                 250                 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
            260                 265                 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
        275                 280                 285

Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
    290                 295                 300

Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
305                 310                 315                 320

Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
                325                 330                 335

Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
            340                 345                 350
```

His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
355 360 365

Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
370 375 380

Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
385 390 395 400

Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
405 410 415

Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
420 425 430

Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
435 440 445

Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
450 455 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
465 470 475 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
485 490 495

Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
500 505 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
515 520 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
530 535 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
545 550 555 560

Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
565 570 575

Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
580 585 590

Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
595 600 605

Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
610 615 620

His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625 630 635 640

Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
645 650 655

Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
660 665 670

His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
675 680 685

```
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
    690                 695                 700

Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
705                 710                 715                 720

Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
                725                 730                 735

Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
            740                 745                 750

Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
        755                 760                 765

Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
    770                 775                 780

Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
785                 790                 795                 800

Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
                805                 810                 815

Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
            820                 825                 830

Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
        835                 840                 845

Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
    850                 855                 860

Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
865                 870                 875                 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
                885                 890                 895

Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
            900                 905                 910

Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
        915                 920                 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
    930                 935                 940

Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
945                 950                 955                 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
                965                 970                 975

Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
            980                 985                 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
        995                 1000                1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
    1010                1015                1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
1025                1030                1035                1040
```

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
1045 1050 1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
1090 1095 1100

Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
1125 1130 1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
1155 1160 1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
1170 1175 1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
1235 1240 1245

Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
1250 1255 1260

Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
1285 1290 1295

Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
1300 1305 1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
1315 1320 1325

Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
1330 1335 1340

Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
1365 1370 1375

```
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
            1380                1385                1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
        1395                1400                1405

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
    1410                1415                1420

Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
1425                1430                1435                1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
                1445                1450                1455

Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
            1460                1465                1470

Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
        1475                1480                1485

Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
    1490                1495                1500

Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
1505                1510                1515                1520

Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
                1525                1530                1535

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
            1540                1545                1550

Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
        1555                1560                1565

Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
    1570                1575                1580

His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
1585                1590                1595                1600

Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
                1605                1610                1615

Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
            1620                1625                1630

Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
        1635                1640                1645

Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
    1650                1655                1660

Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
1665                1670                1675                1680

Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
                1685                1690                1695

Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
            1700                1705                1710

Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
        1715                1720                1725
```

```
Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
    1730                1735                1740

Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
1745                1750                1755                1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
                1765                1770                1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
            1780                1785                1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
        1795                1800                1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
    1810                1815                1820

Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
1825                1830                1835
```

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

```
ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTTACAGT ATTTCTCCGC CGGGTGTAAA      60
ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG     120
GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG     180
GCTGACCCAC AAACTTCAAT AACCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG     240
GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT     300
CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC     360
GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA     420
```

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

```
Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
1               5                   10                  15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
            20                  25                  30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
        35                  40                  45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
    50                  55                  60
```

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
65 70 75 80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
115 120 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
130 135

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 31 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325 330 335

Asn Gln Ala Ala Ala
340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG 60

GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120

GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240

ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

```
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC     360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC     420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC     480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC     540
GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG     600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC     660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC     720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA     780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG     840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG     900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT     960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA    1020
GCCTGA                                                               1026
```

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 33 as follows:

```
TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG      60
CTGAGTGCGC AGATTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG     120
CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC     180
AGGCATAGGC ATTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA     240
ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG     300
AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC     360
TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT     420
GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT     480
AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA     540
CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA     600
CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC     660
TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA     720
GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT     780
```

CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG 840

CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900

TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960

CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020

CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC 1080

CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140

CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200

GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA 1260

CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320

GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380

CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440

CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT 1500

TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560

TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620

CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680

CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1 5 10 15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
180 185 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305 310 315 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
405 410 415

```
Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 35 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                   10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220
```

```
Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335

Gln Ser Thr Ser Thr Gln Pro Met
            340
```

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 36 as follows:

```
ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC    60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC   120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC   180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC   240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC   300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA   360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG   420
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC   480
GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC   540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT   600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC   660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC   720
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG   780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC   840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT   900
```

```
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC     960
GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG    1020
ACGCAGCCGA TGTAA                                                     1035
```

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

```
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1               5                   10                  15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
            20                  25
```

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris pv. pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

```
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1               5                   10                  15

Leu Leu Ala Met
            20
```

Isolation of *Erwinia carotovora* hypersensitive response elictor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma,* and *Phytophora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor. This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," <u>Gene Expression Technology</u> vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., <u>Molecular Cloning: A</u>

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Bacterial Strains and Plasmids

Escherichia coli strains used in the following examples include DH5α and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5α/2139 contained the complete hrpN gene. The 2139 construct was produced by D. Bauer at Cornell University. The hrpN gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated hrpN clones. These clones were subsequently inserted into the $(His)_6$ vector pET28(b) which contained a Kan^r gene for selection of transformants.

<u>Example 2</u> - DNA Manipulation

Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR Supermix™ were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

<u>Example 3</u> - Fragmentation of hrpN Gene

A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length hrpN gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp™ 9700 (Perkin-Elmer, Foster City, CA). 45 μl of Supermix™ (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

H_2O to a final volume of 50 μl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA). Amplified DNA was purified with the QIAquick PCR purification kit, digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 μg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 μl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 μl of ligation solution were added to 100 μl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 μl of transformed cells were placed onto LB agar with 30 μg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden, Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control, and a clone with only the pET28(b) vector was generated as a negative control.

Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins

Escherichia coli BL21(DE3) strains containing the hrpN clones were grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 μg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

37°C to an OD_{620} of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD_{620} of ca. 0.3 or 0.6-0.8. One milli molar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH_2PO_4, and 0.72 K_2HPO_4), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

<u>Example 5</u> - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na_2HPO_4, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated $(His)_6$-binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na_2HPO_4, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na_2HPO_4, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3). The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

Example 6 - Induction of HR in Tobacco

A 1.5 ml aliquot from the 50 ml cultures grown for small scale purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays

Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated $(His)_6$-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

<u>Example 9</u> – Induction of Growth Enhancement (GE)

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked overnight in 10 and 20 μg/ml of the truncated protein diluted with 5mM potassium phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato plants per pot were measured and compared with the heights of the control plants treated only with phosphate buffer. Analyses were done on the heights to determine if there was a significant difference in the height of the plants treated with the truncated proteins compared with the buffer control, and thereby determine whether the proteins induced growth enhancement.

<u>Example 10</u> – Induction of Systemic Acquired Resistance (SAR)

Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves (ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 μg/ml solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and just above the unsprayed leaf) were inoculated with 20 μl of a 1.8 μg/ml solution of TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top surface of the leaves. The TMV entered the plants through tiny lesions made by the diatomaceous earth. Ca. 3-4 days post TMV inolucation, the number of TMV lesions was counted on both leaves compared with the number of lesions on the negative control buffer treated leaves. Analyses were done to determine the efficacy of reducing the number of TMV lesions by the protein fragments compared to the buffer control. Percentage of efficacy was calculated as: Reduction in TMV lesions (% efficacy) = 100 x (1 – mean # of lesions on treated leaves/mean # of lesions on buffer control leaves).

Example 11 - **Expressi n of Hypersensitive Response Elicitor Truncated Proteins**

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large sale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 μg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200μg/ml	-
8	1-75	50μg/ml	-
9	1-104	50μg/ml	3 μg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 μg/ml
13	76-209	2.5 mg/ml	5 μg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10μg/ml
17	105-168	250μg/ml	-
19	137-204	3.6 mg/ml	3.5 μg/ml
20	137-180	250 μg/ml	16 μg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

negative control contained several background proteins as expected, but no obviously induced dominant protein.

Example 12 - **Induction of HR in Tobacco**

The full length positive control protein elicited HR down to only 5-7μg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

One definitive domain encompassing a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

Example 13 – Induction of Plant Growth Enhancement (PGE)

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 μg/ml, but not at the typical 20 μg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 μg/ml	PGE ht>buffer @ 20 μg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14% 16% @ 40μg/ml
6	267-403	21%	21% 23% @ 40μg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60μg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

<u>Example 14</u> – Induction of Systemic Acquired Resistance (SAR)

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

<u>Example 15</u> – Relationship Between HR, PGE, and SAR

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 μg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 μg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

<u>Example 16</u> - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5α, and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD_{620} of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty μl of antifoam and 40 μl of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen μl of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

Table 7

HR test results of HrpZ fragments

HrpZ Fragment	Dilution of Fragment Preparation[a]			
	1:2	1:5	1:25	1:125
152-294	+,+[b]	+,+	+,+	–,–
190-294	–,–	–,–	–,–	–,–

[a] The preparations were diluted with MilliQ water.
[b] The results are indicated for each of two plants. +, HR; –, no HR.

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

HrpZ Fragment	HR elicitation[a]	TMV (% efficacy)[b]	PGE(% > buffer ht)[c]
152-294	+	54.64	4.64
190-294	–	85.25	2.62

[a] +, elicits HR in tobacco leaves; –, no HR in tobacco leaves.
[b] % reduction in TMV lesions in unsprayed leaf of tobacco.
[c] % greater height than buffer sprayed plants.

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H+/Ca2+ influxes, K+/Cl- effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH2-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycroprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

Treatment	Height of seedlings (cm)					Average (cm)	% Change
Buffer	6.0 5.5	6.0 5.5	6.0 5.0	5.5 5.0	5.5 5.5	5.55	-
Peptide Solution (100ng/ml)	6.5 6.0	6.0 6.0	6.5 6.0	6.5 6.0	6.5 6.5	6.25	12.6

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.

2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas, Xanthomonas*, or *Phytophthora*.

3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

9. An isolated fragment according to claim 8, wherein the fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10. An isolated DNA molecule encoding a fragment according to claim 1.

11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas, Xanthomonas*, or *Phythophthora*.

12. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

14. An isolated DNA molecule according to claim 12, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

15. An isolated DNA molecule according to claim 12, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

19. An expression system transformed with a DNA molecule according to claim 10.

20. An expression system according to claim 19, wherein said DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to claim 10.

22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

23. A host cell according to claim 21, wherein the DNA molecule is transformed with an expression system.

24. A transgenic plant transformed with the DNA molecule of claim 10.

25. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

30. A method of imparting disease resistance to plants comprising:

applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

31. A method according to claim 30, wherein plants are treated during said applying.

32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:

applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.

34. A method according to claim 33, wherein plants are treated during said applying.

35. A method according to claim 33, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:

applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated during said applying.

38. A method according to claim 36, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

39. A method of imparting disease resistance to plants comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, and

growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to impart disease resistance.

40. A method according to claim 39, wherein a transgenic plant is provided.

41. A method according to claim 39, wherein a transgenic plant seed is provided.

42. A method of enhancing plant growth comprising:

providing a transgenic plant or a plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, and

growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to enhance plant growth.

43. A method according to claim 42, wherein a transgenic plant is provided.

44. A method according to claim 42, wherein a transgenic plant seed is provided.

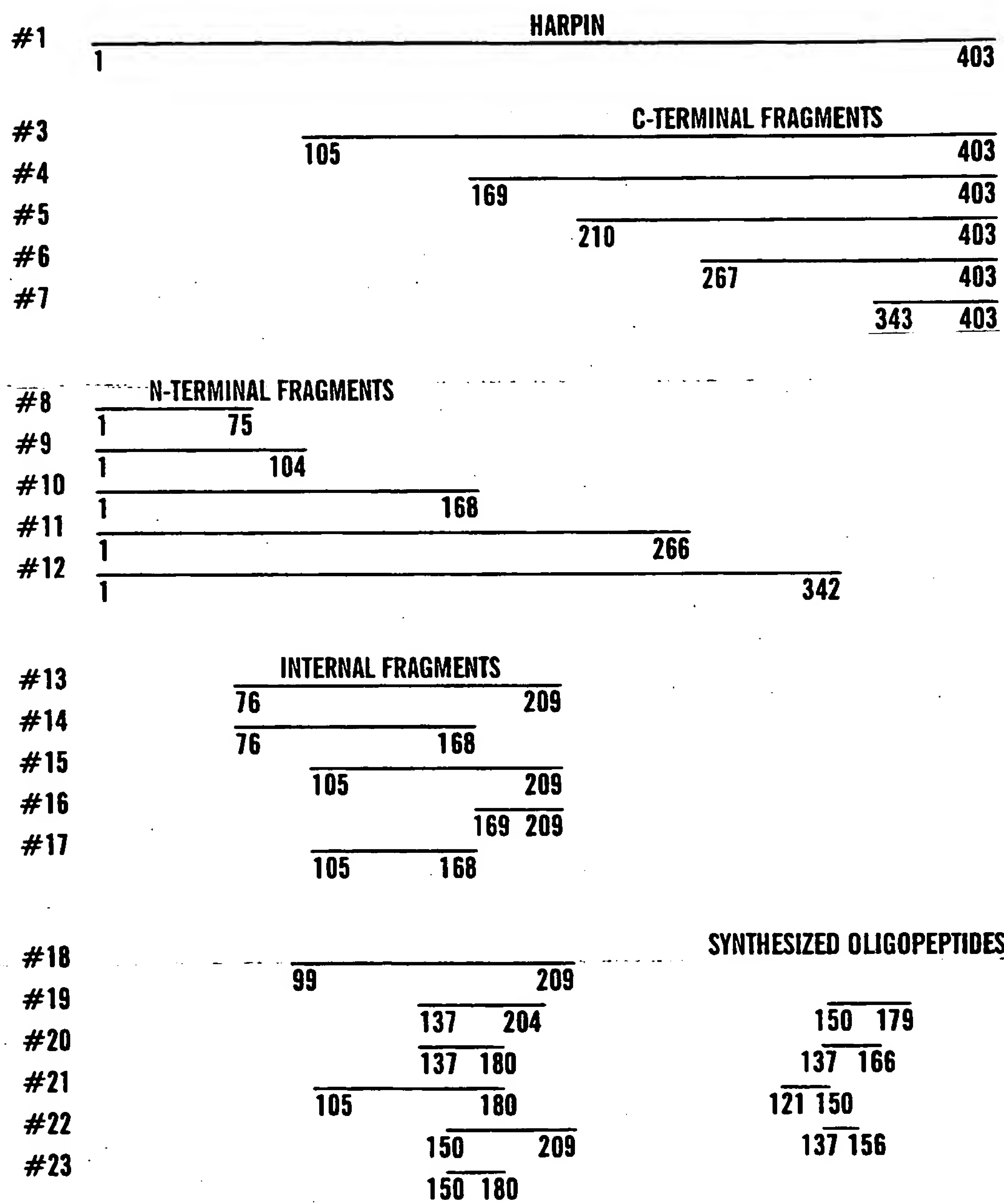
45. A method of insect control for plants comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, and

growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to control insects.

46. A method according to claim 45, wherein a transgenic plant is provided.

47. A method according to claim 45, wherein a transgenic plant seed is provided.



HARPIN FRAGMENTS DERIVED FROM HrpN OF ERWINIA AMYLOVORA

FIG. 1

N1;	5'-GGGAATTCATATGAGTCTGAATACAAGTGGG-3'
N76;	5'-GGGAATTCATATGGGCGGTGGCTTAGGCGGT-3'
N99;	5'-GGCATATGTCGAACGCGCTGAACGATATG-3'
N105;	5'-GGGAATTCATATGTTAGGCGGTTCGCTGAAC-3'
N110;	5'-GGCATATGCTGAACACGCTGGGCTCGAAA-3'
N137;	5'-GGCATATGTCAACGTCCCAAAACGACGAT-3'
N150;	5'-GGCATATGTCCACCTCAGACTCCAGCG-3'
N169;	5'-GGGAATTCATATGCAAAGCCTGTTTGGTGATGGG-3'
N210;	5'-GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'
N267;	5'-GGGAATTCATATGAAAGCGGGCATTCAGGCG-3
N343;	5'-GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'
C75;	5'-GCAAGCTTAACAGCCCACCACCGCCCATCAT-3'
C104;	5'-GCAAGCTTAAATCGTTCAGCGCGTTCGACAG-3'
C168;	5'-GCAAGCTTAATATCTCGCTGAACATCTTCAGCAG-3'
C180;	5'-GCAAGCTTAAGGTGCCATCTTGCCCATCAC-3'
C204;	5'-GCAAGCTTAAATCAGTGACTCCTTTTTTATAGGC-3
C209;	5'-GCAAGCTTAACAGGCCCGACAGCGCATCAGT-3'
C266;	5'-GCAAGCTTAAACCGATACCGGTACCCACGGC-3'
C342;	5'-GCAAGCTTAATCCGTCGTCATCTGGCTTGCTCAG-3'
C403;	5'-GCAAGCTTAAGCCGCGCCCAGCTTG-3'

OLIGONUCLEOTIDE PRIMERS USED FOR THE CONSTRUCTION
OF THE SUBCLONES OF ERWINIA AMYLOVORA HrpN

FIG. 2

SEQUENCE LISTING

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<213> Erwinia chrysanthemi

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Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
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Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
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Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
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Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115 120 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130 135 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145 150 155 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165 170 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180 185 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195 200 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210 215 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225 230 235 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275 280 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr

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<213> Erwinia chrysanthemi

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35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
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Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
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Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser S r Gly Gly Lys Gln Pro Thr Glu
180 185 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
210 215 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
245 250 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
275 280 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
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Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
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Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
325 330 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
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<212> DNA
<213> Erwinia amylovora

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<212> DNA
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Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
35 40 45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
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Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
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Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
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Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
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Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
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Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
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Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
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Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
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Gly Gly Lys Ala Pro Ser Gly Asn Ser Pr Ser Gly Asn Tyr Ser Pro
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Val Ser Thr Phe Ser Pro Pro Ser Thr Pr Thr Ser Pr Thr Ser Pr
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Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
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Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
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Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
340 345 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
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Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
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Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
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His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
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<212> PRT
<213> Erwinia amylovora

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Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
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Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
180 185 190

Arg Leu Gln His Ser Pr Pro His Ile Pro Gly Ser His His Glu Ile

195 200 205

Lys Glu Glu Pr Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
210 215 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
225 230 235 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
245 250 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
260 265 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
275 280 285

Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
290 295 300

Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
305 310 315 320

Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
325 330 335

Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
340 345 350

His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
355 360 365

Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
370 375 380

Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
385 390 395 400

Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
405 410 415

Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
420 425 430

Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
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Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp

450 455 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
465 470 475 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
485 490 495

Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
500 505 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
515 520 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
530 535 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
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Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
565 570 575

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580 585 590

Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
595 600 605

Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
610 615 620

His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625 630 635 640

Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
645 650 655

Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
660 665 670

His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
675 680 685

Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
690 695 700

Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly

705 710 715 720

Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
725 730 735

Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
740 745 750

Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
755 760 765

Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
770 775 780

Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
785 790 795 800

Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
805 810 815

Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
820 825 830

Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
835 840 845

Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
850 855 860

Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
865 870 875 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
885 890 895

Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
900 905 910

Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
915 920 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
930 935 940

Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
945 950 955 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr

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Met Ser Thr Pro Arg Pr Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
980 985 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
995 1000 1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
1010 1015 1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
1025 1030 1035 1040

~~Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu~~
1045 1050 1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
1090 1095 1100

Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
1125 1130 1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
1155 1160 1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
1170 1175 1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp

1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
1235 1240 1245

Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
1250 1255 1260

Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
1285 1290 1295

Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
1300 1305 1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
1315 1320 1325

Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
1330 1335 1340

Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
1365 1370 1375

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
1380 1385 1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
1395 1400 1405

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
1410 1415 1420

Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
1425 1430 1435 1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
1445 1450 1455

Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
1460 1465 1470

Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg

1475 1480 1485

Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
1490 1495 1500

Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
1505 1510 1515 1520

Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
1525 1530 1535

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
1540 1545 1550

Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
1555 1560 1565

Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
1570 1575 1580

His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
1585 1590 1595 1600

Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
1605 1610 1615

Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
1620 1625 1630

Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
1635 1640 1645

Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
1650 1655 1660

Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
1665 1670 1675 1680

Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
1685 1690 1695

Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
1700 1705 1710

Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
1715 1720 1725

Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val

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Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
1745 1750 1755 1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
1765 1770 1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
1780 1785 1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
1795 1800 1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
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<213> Erwinia amylovora

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gcggtgctgg aagtaccgca acacagcgac agcctgttac tacactgccg aatcattgag 180
gctgacccac aaacttcaat aaccctgtat tcgatgctat tacagctgaa ttttgaaatg 240
gcggccatgc gcggctgttg gctggcgctg gatgaactgc acaacgtgcg tttatgtttt 300
cagcagtcgc tggagcatct ggatgaagca agttttagcg atatcgttag cggcttcatc 360
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<213> Erwinia amylovora

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20 25 30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His

35 40 45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Il Glu Ala Asp Pro Gln
50 55 60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
65 70 75 80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
115 120 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
130 135

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<212> PRT
<213> Pseudomonas syringae

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20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro M t
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325 330 335

Asn Gln Ala Ala Ala
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<213> Pseudomonas syringae

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gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc 480
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<213> Pseudomonas syringae

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cctctgagtg cggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc 180
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aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga 540
cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
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taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720
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tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc 960
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caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca 1680
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<212> PRT
<213> Pseudomonas syringae

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Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly

165 170 175

Ser Gly Gly Thr Pr Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
180 185 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305 310 315 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
405 410 415

Ala Ser Thr Gln His Thr Glu Leu

420

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<211> 344
<212> PRT
<213> Pseudomonas solanacearum

<220>
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Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
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INTERNATIONAL SEARCH REPORT

Inte ' Application No
PCT/US 99/23181

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/195 C12N15/31 C12N1/21 C12N5/10 A01H5/00
A01H5/10 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NÜRNBERGER T, ET AL. : "High Affinity Binding of a Fungal Oligopeptide Elicitor to arsley Plasma Membranes Triggers Multiple Defense Responses" CELL, vol. 78, no. 3, 12 August 1994 (1994-08-12), pages 449-460, XP000882736 Cambridge, Mass. cited in the application the whole document	1,2,10, 11, 19-23, 30-32, 36-38
A	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) the whole document	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 March 2000	03/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bilang, J

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) the whole document	
A	WEI Z-M, ET AL.: "Harpin, an HR elicitor, activates both defense and growth systems in many commercially important crops" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S96 XP000882741 abstract	
A	NIGGEMEYER J, ET AL.: "Characterization of the functional domains of harpin" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S67 XP000882740 abstract	

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INTERNATIONAL SEARCH REPORT
In...ation on patent family members

Inter... Application No
PCT/US 99/23181

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9832844 A	30-07-1998	AU 6043198 A	18-08-1998
WO 9824297 A	11-06-1998	AU 5693598 A	29-06-1998
		EP 0957672 A	24-11-1999

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WO 01/80639 A2

(54) Title: TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION

(57) Abstract: The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable, either by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation, or by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant. Also disclosed are DNA constructs and expression systems, host cells, and transgenic plants containing the DNA construct.

TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/198,359, filed April 19, 2000, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods of treating fruits or vegetables to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

BACKGROUND OF THE INVENTION

Postharvest diseases are often extensions of disease occurring in the field or orchard. Brown rot of stone fruits (*Monilinia fructicola* (Wint.) Honey), for example, may cause blossom and twig blighting in the orchard. Infections in the orchard may not be visible at harvest if fruits are not refrigerated. *Colletotrichum gloeosporioiders* (Penz.) Arx may attack blossoms or leaves and young fruit of citrus, avocados, mangos, papayas, and a wide range of other tropical and subtropical species; infections in developing fruit are usually latent, and rot lesions appear only at the onset of fruit ripening. *Pezicula malicorticis* (Jacks.) Nannfld. causes cankers of limbs of apples and pears; infections in developing fruit are latent, and active rotting usually commences only after the fruit has spent several months in storage and proceeds during -1°C storage because the organism is able to grow at very low temperatures. These fungi used as examples are able to penetrate the cuticle and epidermis of the fruit.

Whether capable of being penetrated directly or not, wounds are often the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and abrasions cannot be avoided completely during harvest and handling. If the cuticle and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for spore germination and colonization. Separation of fruits from the parent plant at harvest creates an unavoidable wound that encourages stem-end rots.

Rots developing at the blossom end usually involve prior colonization of floral parts. For example, *Botrytis* blossom-end rot (*B. cinerea*) sometimes occurs in Bartlett pears after a month or two in storage at -1°C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by *Alternaria* spp. and common saprophytic fungi, but *B. cinerea* also is found occasionally. Not all fruits having *B.cinerea*-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from *Botrytis* blossom-end rot if the old floral parts of developing fruits are free from *B. cinerea*. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to

processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be effective in biologically controlling postharvest pathogens.

Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp. 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of *Botrytis* Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was controlled with *Bacillus subtilis* (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of *Trichoderma* (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by *Geotrichum candidum* and *Penicillium digitatum*," Ph.D. dissertation, University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with *Bacillus subtilis* (Singh et al., "*Bacillus subtilis* as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or competition for nutrients and space or both, induction of resistance in the host tissue, and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae, Pseudomonas solanacearum,* etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitins) from various *Phytophthora* species (e.g., *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma, Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi, Erwinia amylovora, Pseudomonas syringae,* or *Pseudomonas solanacearum*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15
Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30
Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80
```

```
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

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ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccctttag 1920
cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctgggcga cctgatcggt t                     2141
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

```
Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                   10                  15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30
Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
        35                  40                  45
Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
    50                  55                  60
Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65                  70                  75                  80
Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
                85                  90                  95
Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
            100                 105                 110
Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
        115                 120                 125
Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
    130                 135                 140
Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145                 150                 155                 160
Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
                165                 170                 175
Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
            180                 185                 190
Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
        195                 200                 205
Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
    210                 215                 220
Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225                 230                 235                 240
Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
                245                 250                 255
Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
            260                 265                 270
Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
        275                 280                 285
Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
    290                 295                 300
Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305                 310                 315                 320
Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
                325                 330                 335
```

```
Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
            340                 345                 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
        355                 360                 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                 375                 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                 390                 395                 400

Gly Ala Ala
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa    60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct   120
atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg   180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg   240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggtgg tgggctgatg   300
ggcggtggct taggcggtgg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa   360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa   420
ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggtattaac   480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac   540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg   600
caagatggca cccagggcag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac   660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag   720
ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggtcttgac   780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag   840
ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat   900
atcggtacgc acaggcacag ttcaacccgt tctttcgtca ataaaggcga tcgggcgatg   960
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac  1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc  1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc  1140
```

```
atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc   1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca   1260
cttggcaagc tgggcgcggc ttaagctt                                      1288
```

The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                   10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                  80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255
```

```
Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
        260                 265                 270
Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
    275                 280                 285
Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300
Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320
Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335
Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350
Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365
Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380
Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400
His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415
Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430
Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg    60
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat   120
cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg   180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt   240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccactccgca gtctgacagt   300
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc   360
cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca   420
cgcatgatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc   480
tcttccggta cttcttcatc tggcggttcc ccttttaacg atctatcagg ggggaaggcc   540
ccttccggca actcccctte cggcaactac tctcccgtca gtaccttctc acccccatcc   600
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg   660
```

ggcagcacgc cggtaaccga tcatcctgac cctgttggta gcgcgggcat cggggccgga 720
aattcggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840
ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc 900
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcatcttta cggtgatgcc 960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020
agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140
tttggtactt ttgtacgcac taacggcggt caacagggta actgggatct gaatctgagc 1200
catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320
~~gccaacctga aggtggctga atga~~ ~~1344~~

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,927 to Beer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

```
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

```
atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccc cggcaatggc ccttgtcctg    60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc   120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga   180
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc   240
atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg   300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc   360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac   420
gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc   480
```

```
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac    540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag    600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc    660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc    720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa    780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg    840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag    900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct    960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca   1020
gcctga                                                              1026
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                   10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190
```

```
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285
Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300
Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320
Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335
Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350
Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365
Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380
Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400
Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415
Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg     60
ctgagtgcgc agatttcgtt gataagggtg tggtactggt cattgttggt catttcaagg    120
cctctgagtg cggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc    180
aggcataggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa    240
acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg    300
agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc    360
tctggtaaac cctggagctg gcgtcggtcc aattgcccac ttagcgaggt aacgcagcat    420
gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccactcgatt tttcggcgct    480
```

```
aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga    540
cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga    600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc    660
taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa    720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct    780
cggtacaccg tcggccgata gcgggggcgg cggtacaccg gatgcgacag gtggcggcgg    840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg    900
tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc    960
cactgcaaca ggcggtggcg agggtggcgt aacaccgcaa atcactccgc agttggccaa   1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc   1080
cggcaagatc aatgtggtga aagacaccat caaggtcggc gctggcgaag tctttgacgg   1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca   1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa   1260
cgaggtcgat ggcatccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt   1320
gcatgcccag aacgtcggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt   1380
cactaatctg aacatcaaga acagcagtgc caaaggtgca gacgacaagg ttgtccagct   1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt   1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc   1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg   1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca   1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc              1729
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,817 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                   10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95
```

```
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125
Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140
Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160
Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175
Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190
Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205
Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220
Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240
Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255
Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270
Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285
Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300
Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320
Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335
Gln Ser Thr Ser Thr Gln Pro Met
            340
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Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

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atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc    60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc   120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc   180
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ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc    240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc    300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa    360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag    420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc    480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc    540
ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt    600
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac    660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac    720
cagggcggcc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag    780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc    840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat    900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc    960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg   1020
acgcagccga tgtaa                                                    1035
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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et

al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in

its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the

protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*). Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, or 12. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions

or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 μg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which

is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,

which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not

limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

The methods of the present invention can be performed by treating the fruit or vegetable either prior to or after harvest of the fruit or vegetable.

Suitable preharvest application methods include, without limitation, high or low pressure spraying of the entire plant and fruits. Suitable postharvest application methods include, without limitation, low or high pressure spraying, coating, or immersion. Other suitable application procedures (both preharvest and postharvest) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with the fruit or vegetable. Once treated, the fruits or vegetables can be handled, packed, shipped, and processed using conventional procedures to deliver the produce to processing plants or end-consumers.

The hypersensitive response elicitor polypeptide or protein can be applied to fruits or vegetables in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to fruits or vegetables with other materials being applied at different times.

A composition suitable for treating fruits or vegetables in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit postharvest disease and desiccation.

As indicated above, one embodiment of the present invention involves treating fruits or vegetables with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora, Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide..

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in fruit or vegetable

tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a heterologous DNA sequence in transgenic tomato plants (Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference in its entirety). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter

described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

~~Promoters useful for expression in tubers, particularly potato tubers,~~ include the patatin promoter.

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gst1* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an

antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control or a promoter that induces transcription of the DNA molecule fruit or vegetable tissues. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With regard to the use of the hypersensitive response elicitor protein or polypeptide in imparting postharvest disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease can be reduced and symptom development can be delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of controlling postharvest disease has the potential for controlling previously untreatable diseases and avoiding the use of infectious agents or environmentally harmful materials.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., use of coating waxes) which may contribute to reduced costs or, at least, substantially no increase in costs.

The methods of the present invention can be used to control a number of postharvest diseases caused by a variety of pathogens. These postharvest diseases and the causative agents which can be treated according to the present invention include, without limitation, the following: *Penicillium* (e.g., *Penicillium digitatum*), *Botrytis* (e.g., *Botrytis cinereaon*), *Phytophthora* (e.g., *Phytophthora citrophthora*), and *Erwinia* (e.g. *Erwinia carotovora*).

A further aspect of the present invention relates to a method of enhancing the longevity of fruit or vegetable ripeness.

According to one embodiment, this aspect of the present invention is carried out by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness. Preferably, as noted above, the hypersensitive response elicitor protein or polypeptide is in isolated form. Treating of the fruit or vegetable can be performed either prior to harvest after harvest of the fruit or vegetable, using the techniques described above.

According to another embodiment, this aspect of the present invention is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and then growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant. This aspect of the present invention may further include applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness. Treating of the fruit or vegetable can be performed either prior to harvest or after harvest of the fruit or vegetable, using the techniques described above.

The methods of the present invention can be utilized to treat a wide variety of fruits and vegetables to control postharvest disease or desiccation as well as enhance the longevity of fruit or vegetable ripeness. Fruits and vegetables which can be treated include any edible plant product, particularly those from traditional crop plants, such as seed, root, tuber, stem, leaf, flower, and fruit. Exemplary transgenic fruit plants and fruits that can be treated include, without limitation, apple, pear, peach, nectarine, apricot, plum, cherry, olive, melon, citrus, grape, strawberry, raspberry, blueberry, currant, pineapple, papaya, guava, banana, and kiwi. Exemplary transgenic vegetable plants and vegetables that can be treated include, without limitation, asparagus, potato, sweet potato, bean, pea, chicory, lettuce, parsley, basil, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, spinach, onion, garlic, eggplant, pepper, celery, leek, radish, carrot, squash, pumpkin, zucchini, cucumber, soybean, tobacco, tomato, sorghum, rhubarb, and sugarcane. Exemplary transgenic grain plants and grain products which can be treated include, without limitation, alfalfa, rice, wheat, barley, corn, and rye.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

As used in the following Examples, Messenger® refers to a product available from Eden Bioscience Corporation (Bothell, Washington), which contains 3% by weight of harpin$_{Ea}$ as the active ingredient and 97% by weight inert ingredients. Harpin$_{Ea}$ is one type of hypersensitive response elicitor protein from *Erwinia amylovora,* identified herein by SEQ. ID. No. 3.

Example 1 – Effect of Treating Orange Fruits with Messenger® on Postharvest Orange Storage

On day 0, Fall-GLO orange fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on oranges were then dried by air, and the treated oranges were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated oranges was then put into a 18°C growth chamber for storage. On day 7, orange fruits were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of orange fruit. The above procedure was performed on 40 orange fruits per treatment.

Measurements of disease were conducted on days 20, 24, and 26 following treatment with Messenger® or buffer solution. Grades 0-5 indicate different disease scales -- Grade 0: No symptoms; Grade 1: 1/5 an individual fruit has disease symptoms; Grade 2: 2/5 an individual fruit has disease symptoms; Grade 3: 3/5 an individual fruit has disease symptoms; Grade 4: 4/5 an individual fruit has disease symptoms; Grade 5: whole fruit has disease symptoms. The results of these treatments are set forth in Table 1 below.

Table 1: Reduction of Disease Index in Oranges

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	20	33	3	1	0	2	1	0.09	58.14%	yes	yes
Buffer	20	23	8	0	2	6	1	0.22	n/a	-	-
Messenger®	24	25	2	6	4	1	2	0.20	45.21%	yes	yes
Buffer	24	16	7	3	3	4	7	0.37	n/a	-	-
Messenger®	26	19	4	6	4	5	2	0.29	36.96%	yes	yes
Buffer	26	16	3	3	0	7	11	0.46	n/a	-	-

The data listed in Table 1 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce orange disease about 58.14% at 21 days, about 45.21% at 25 days, and 36.97% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95%

and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 2 – Effect of Treating Tomato (Hot House) Fruits with Messenger® on Postharvest Tomato Storage

On day 0, Hot House tomato fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on tomatoes were then dried by air, and the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of tomato fruit. The above procedure was performed on 15 tomatoes fruits per treatment.

Measurements of disease were conducted on days 21 and 27 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 2 below.

Table 2: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	21	7	2	2	3	1	0	0.25	58.70%	yes	yes
Buffer	21	3	1	2	1	2	6	0.61	n/a	-	-
Messenger®	27	2	2	4	3	2	2	0.49	30.19%	yes	yes
Buffer	27	1	1	2	2	3	6	0.71	n/a	-	-

The data listed in Table 2 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce tomato disease about 58.70% at 21 days and about 30.19% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 3 – Effect of Treating Grape Fruits with Messenger® on Postharvest Grape Storage

On day 0, Red G. Grape fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapes were then dried by air, and the treated grapes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapes was then put into a 18°C growth chamber for storage. On day 7, grapes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of grape fruit. The above procedure was performed on about 3700g of grape fruits per treatment.

Measurements of disease were conducted on days 14 and 21 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 3 below.

Table 3: Reduction of Disease Index in Grapes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	14	225	99	42	39	21	13	0.20	45.65%	yes	yes
Buffer	14	98	130	91	52	38	48	0.38	n/a	-	-
Messenger®	21	66	83	126	98	39	27	0.42	39.35%	yes	yes
Buffer	21	18	36	64	72	119	137	0.69	n/a	-	-

The data listed in Table 3 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce grape disease by about 45.65% at 14 days and about 39.35% at 21 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 4 – Effect of Treating Grapefruit Fruits with Messenger® on Postharvest Grapefruit Storage

On day 0, FL 33935 grapefruit fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapefruits were then dried by air, and the treated grapefruits were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapefruit fruits was then put into a 18°C growth chamber for storage. On day 7, grapefruit fruits were inoculated with *Phytophthora citrophthora* by spraying a 10^5 cfu/ml suspension on the surface of grapefruit fruit. The above procedure was performed on 6 grapefruit fruits per treatment.

Measurements of disease were conducted on days 87, 97, 103, and 111 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 4 below.

Table 4: Reduction of Disease Index in Grapefruits

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	87	5	1	0	0	0	0	0.03	75.00%	yes	yes
Buffer	87	4	1	0	1	0	0	0.13	n/a	-	-
Messenger®	97	5	0	0	1	0	0	0.10	50.00%	yes	yes
Buffer	97	4	0	1	0	1	0	0.20	n/a	-	-
Messenger®	103	4	1	0	0	1	0	0.17	28.57%	yes	yes
Buffer	103	3	2	0	0	0	1	0.23	n/a	-	-
Messenger®	111	4	1	0	0	0	1	0.20	33.33%	yes	yes
Buffer	111	3	1	0	1	0	1	0.30	n/a	-	-

The data listed in Table 4 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Phytophthora citrophthora* and providing longer storage life. Messenger® treatment can reduce grapefruit disease by about 75.00% at 87 days, about 50.00% at 97 days, about 28.57% at 103 days, and about 33.33% at 111 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant

differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 5 - **Effect of Treating Apple (Fuji) Fruits with Messenger® on Postharvest Apple Storage**

On day 0, Fuji apple fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10^5 cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 5 below.

Table 5: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	50	20	0	0	0	0	0	0.00	100.00%	yes	yes
Buffer	50	18	1	1	0	0	0	0.03	n/a	-	-
Messenger®	61	19	1	0	0	0	0	0.01	88.89%	yes	yes
Buffer	61	16	2	1	0	0	1	0.09	n/a	-	-
Messenger®	70	18	0	2	0	0	0	0.04	71.43%	yes	yes
Buffer	70	14	2	2	1	0	1	0.14	n/a	-	-
Messenger®	78	15	2	3	0	0	0	0.08	57.89%	yes	yes
Buffer	78	13	2	2	1	0	2	0.19	n/a	-	-
Messenger®	85	13	3	1	1	2	0	0.16	40.74%	yes	yes
Buffer	85	10	5	0	0	3	2	0.27	n/a	-	-

The data listed in Table 5 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life.

Messenger® treatment can reduce apple disease by about 100.00% at 51 days, 88.89% at 61 days, 71.43% at 70 days, 57.89% at 78 days, and 40.74% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

<u>Example 6</u> - Effect of Treating Apple (Granny Smith) Fruits with Messenger® on Postharvest Apple Storage

On day 0, Granny Smith apple fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10^5 cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 6 below.

Table 6: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	50	20	0	0	0	0	0	00.00	100.00%	yes	yes
Buffer	50	19	1	0	0	0	0	0.01	n/a	-	-
Messenger®	61	13	5	2	0	0	0	0.09	50.00%	yes	yes
Buffer	61	7	9	3	1	0	0	0.18	n/a	-	-
Messenger®	70	7	10	3	0	0	0	0.16	36.00%	yes	yes
Buffer	70	2	12	5	1	0	0	0.25	n/a	-	-
Messenger®	78	6	10	3	1	0	0	0.19	32.14%	yes	yes
Buffer	78	2	11	5	1	1	0	0.28	n/a	-	-
Messenger®	85	7	9	2	1	1	0	0.20	23.08	yes	yes
Buffer	85	4	10	4	1	0	1		n/a	-	-

The data listed in Table 6 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life. Messenger® treatment can reduce apple disease by about 100.00% at 51 days, 50.00% at 61 days, 36.00% at 70 days, 32.14% at 78 days, and 23.08% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

Example 7 - Effect of Treating Tomato Fruits with Messenger® on Postharvest Tomato Storage

On day 0, tomato fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO_4, pH 6.8) on the surface of fruits in a 22°C greenhouse. After the Messenger® or buffer solutions on tomatoes were dried by air, the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into a 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinereaon* by spraying a 10^5 cfu/ml suspension on the surface of tomatoes. The above procedure was performed on 44 tomatoes per treatment.

Measurements of disease were conducted on days 18, 27, 35, and 42 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 7 below.

Table 7: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade 0	1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
Messenger®	18	21	18	5	0	0	0	0.13	37.78%	yes	yes
Buffer	18	11	21	12	0	0	0	0.20	n/a	-	-
Messenger®	27	16	18	9	1	0	0	0.18	25.00%	yes	yes
Buffer	27	8	24	8	4	0	0	0.24	n/a	-	-
Messenger®	35	7	14	13	10	0	0	0.32	16.67%	yes	yes
Buffer	35	1	16	15	10	2	0	0.38	n/a	-	-
Messenger®	42	1	10	9	12	9	3	0.52	12.88%	yes	yes
Buffer	42	0	3	15	10	11	5	0.60	n/a	-	-

The data listed in Table 7 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinereaon* and providing longer storage life. Messenger® treatment can reduce tomato disease by about 37.78% at 18 days, 25.00% at 27 days, 16.67% at 35 days, and 12.88% at 42 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger® treatment and buffer treatment.

<u>Example 8</u> - Effect of Preharvest and Postharvest Messenger® Treatments on Tomato (Sanibel) Fruit Postharvest Storage

Plots of red and green Sanibel variety tomatoes were grown under either standard conditions or full Messenger® treatment over the course of the growing season. The standard conditions, also known as grower's standard, included fungicide treatment sprayed every seven days after transplanting using primarily fungicides containing copper-based active ingredients. The Messenger® treatment included six sprays at rate of 2.2 oz of the product per acre.

Red and green fruits were harvested from both the Messenger® treated and grower standard plots. It was noted that green tomatoes from the grower standard treatment plots were smaller (i.e. less mature) then green tomatoes from the messenger treated plants.

Harvested fruits were treated as follows:

(1) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(2) Fruits from standard plots were treated with Messenger® after harvest;

(3) Fruits from Messenger® treated plots received no additional treatment following harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (1) and (2) was carried out by spraying with Messenger® at a rate of 20 ppm $harpin_{Ea}$ concentration using a

backpack-sprayer at about 30 p.s.i. The fruit were rolled during application to assure full coverage of the spray. The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in storage in a single layer. Storage temperatures ranged from about 18 to 32°C and light intervals were approximately 12 hours of light and darkness. Tomatoes were checked daily for rot and desiccation for a total of 31 days after harvest. The results are shown in Table 8 below.

Table 8: Affect of Preharvest and Postharvest Treatment on Rot and Desiccation

Group	Ripeness	No. Fruit	Days After Harvest 14	19	21	22	23	25	31	No. Desiccated	% Marketable
(1) Pre/Postharvest Messenger®	Red	5	0	0	0	1	1	1	2	0	60%
	Green	4	0	0	0	0	0	0	0	0	100%
(2) Postharvest Messenger® Only	Red	5	0	0	0	0	0	0	0	4	20%
	Green	4	0	0	0	0	0	0	0	1	75%
(3) Preharvest Messenger® Only	Red	5	0	0	0	0	0	0	2	0	60%
	Green	5	0	0	0	0	0	0	0	0	100%
(4) No Messenger®	Red	5	1	3	1	5	5	5	5	0	0%
	Green	5	0	0	0	0	0	0	0	1	80%

The red tomatoes from group (4) all rotted by day 21. In contrast, all red tomatoes which received some form of Messenger® treatment showed reduced rate of decay and rot. Near the end of the trial a number of tomatoes were observed to have desiccated, exhibiting shriveled skins but no rot. These were included as non-marketable. These results are suggestive that both preharvest and postharvest Messenger® treatments can reduce the level of rotting and desiccation, thereby extending fresh storage time.

Example 9 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato During Ambient Storage

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Three different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from standard plots were treated with Messenger® after harvest;

(3) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from group (2) was carried out by dipping the fruit in a Messenger® solution (20 ppm $harpin_{Ea}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(3) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the rating scale below.

Grade	Description
1	Mature Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
2	Pink: Initial sign of color break noticed on some areas of fruit; these areas are usually pink;
3	Pink/Red: Intermediate ripening: Fruit is not total red; some pink still remains;
4	Red: Fruit totally red in color;
5	Decay: Some areas of the fruit beginning to break down from decay.

The results of this test are summarized in Table 9 below.

Table 9: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	10	11	6	8	75	0	0.69	7.28%	yes	yes
2	10	5	7	11	77	0	0.72	3.81%	yes	yes
3	10	5	3	6	86	1	0.75	N/A	N/A	N/A
1	14	4	5	5	86	0	0.75	2.61%	yes	yes
2	14	2	6	5	87	0	0.75	1.57%	yes	yes
3	14	2	4	4	89	1	0.77	N/A	N/A	N/A
1	17	0	0	3	92	5	0.80	3.37%	yes	yes
2	17	0	1	4	82	13	0.81	2.16%	yes	yes
3	17	0	0	1	82	17	0.83	N/A	N/A	N/A
1	20	0	0	0	89	11	0.82	2.61%	yes	yes
2	20	0	0	0	80	20	0.84	0.47%	yes	yes
3	20	0	0	1	76	23	0.84	N/A	N/A	N/A

The data generated in this trial indicate that treatment of tomatoes with Messenger®, either through field sprays or as a post harvest dip, resulted in earlier fruit red ripening compared to grower's standard. In addition, although early ripening was observed, the Messenger® treatments maintained the red ripe condition longer than the grower's standard with delay of breakdown and decay.

Example 10 - **Effect on Messenger on Post Harvested Maturity and Fruit Decay of Tomato Under Cold Storage Conditions**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(3) Fruits from standard plots were treated with Messenger® after harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm $harpin_{Ea}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage in a Custom Packing House cooler at 11°C (52°F). The tomatoes were then rated for color development and decay over time using the rating scale described in Example 8. The results of this study appear in Table 10 below.

Table 10: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	7	66	34	0	0	0	0.27	0.00%	yes	yes
2	7	67	33	0	0	0	0.27	0.75%	yes	yes
3	7	76	24	0	0	0	0.27	7.46%	yes	yes
4	7	68	30	2	0	0	0.27	N/A	yes	yes
1	10	59	31	8	0	0	0.30	7.53%	yes	yes
2	10	60	28	12	0	0	0.30	5.00%	yes	yes
3	10	65	35	0	0	0	0.27	15.63%	yes	yes
4	10	49	42	9	0	0	0.32	N/A	N/A	N/A
1	17	19	35	28	18	0	0.49	7.20%	yes	yes
2	17	20	38	28	14	0	0.47	10.61%	yes	yes
3	17	19	28	39	14	0	0.50	6.06%	yes	yes
4	17	17	27	31	25	0	0.53	N/A	N/A	N/A
1	21	11	28	29	32	0	0.56	6.62%	N/A	N/A
2	21	15	26	37	22	0	0.53	11.92%	yes	yes
3	21	10	33	35	22	0	0.54	10.93%	yes	yes
4	21	10	18	32	40	0	0.60	N/A	N/A	N/A
1	26	3	15	23	59	0	0.68	-2.26%	yes	yes
2	26	9	19	25	41	6	0.63	4.39%	yes	yes
3	26	3	23	31	43	0	0.63	5.00%	yes	yes
4	26	2	19	23	50	1	0.66	N/A	N/A	N/A
1	32	3	15	23	59	0	0.68	-2.26%	yes	yes
2	32	9	19	25	41	6	0.63	4.39%	yes	yes
3	32	3	23	31	43	0	0.63	5.00%	yes	yes
4	32	2	19	23	50	1	0.66	N/A	N/A	N/A

Table 10 cont.

Group	Days After Treatment	Grade 1	2	3	4	5	Index	Efficacy	T-test p<0.05	p<0.01
1	38	0	4	10	84	2	0.77	0.26%	yes	yes
2	38	1	10	15	65	9	0.74	3.64%	yes	yes
3	38	1	5	14	78	2	0.75	2.60%	yes	yes
4	38	0	3	13	80	4	0.77	N/A	N/A	N/A
1	45	0	3	11	74	12	0.79	2.95%	yes	yes
2	45	1	4	12	69	14	0.78	3.93%	yes	yes
3	45	0	1	11	81	7	0.79	3.19%	yes	yes
4	45	0	0	10	73	17	0.81	N/A	N/A	N/A
1	50	0	3	10	63	23	0.82	3.55%	yes	yes
2	50	0	4	11	58	27	0.82	3.55%	yes	yes
3	50	0	0	8	78	14	0.81	4.02%	yes	yes
4	50	0	0	3	71	26	0.85	N/A	N/A	N/A
1	55	0	0	0	73	27	0.85	1.84%	yes	yes
2	55	0	0	0	68	32	0.86	0.69%	yes	yes
3	55	0	0	2	80	18	0.83	4.37%	yes	yes
4	55	0	0	0	65	35	0.87	N/A	N/A	N/A
1	60	0	0	0	65	35	0.87	2.47%	yes	yes
2	60	0	0	0	63	37	0.87	2.02%	yes	yes
3	60	0	0	0	74	26	0.85	4.48%	yes	yes
4	60	0	0	0	54	46	0.89	N/A	N/A	N/A
1	65	0	0	0	53	47	0.89	1.76%	yes	yes
2	65	0	0	0	58	42	0.88	2.86%	yes	yes
3	65	0	0	0	65	35	0.87	4.40%	yes	yes
4	65	0	0	0	45	55	0.91	N/A	N/A	N/A

In previous trials when tomatoes were treated with Messenger® in the field and/or with a post harvest dip, the fruit appeared to develop to red ripe more quickly than the grower's standard, when held at ambient temperatures (75-80°F). Although this early ripening was observed, these red fruit did not begin to decay earlier than the grower's standard. In this study, the fruit were held at a constant 52°F in a commercial cold storage room at a tomato packinghouse facility. It appears that this lower temperature slows the ripening process, as would be expected, and Messenger® treatments did not increase the rate of the red ripening for the first 30 days, as observed in previous tests. The Messenger® treatments did, however,

seem to maintain the red ripe condition longer than the grower's standard without breakdown and decay.

Example 11 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

(1) Fruits from Messenger® treated plots received no additional treatment following harvest;

(2) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

(3) Fruits from standard plots were treated with Messenger® after harvest; and

(4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm harpin$_{Ea}$). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the commercial rating scale from the Florida Tomato Committee color guide as follows:

Grade	Description
1	Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
2	Breakers: Initial sign of color break on 10% or less of the area of fruit; these areas are usually pink;
3	Turning: Pink or red on 10 to 30% of the fruit surface;

4 Pink: Pink or red on 30 to 60% of the fruit surface;

5 Light Red: Pink on over 60% of fruit surface and red color no more than 90% of fruit surface;

6 Red: Fruit totally red in color; and

7 Decay: Some areas of the fruit beginning to break down from decay.

The results of this treatment are set forth in Table 11 below.

Table 11: Affect of Preharvest and Postharvest Treatment on Maturity and Decay Data

Group	Days After Treatment	Grade 1	2	3	4	5	6	7	Index	Efficacy	T-test p<0.05	p<0.01
1	3	80	18	2	0	0	0	0	0.17	0.00%	no	no
2	3	73	17	9	1	0	0	0	0.20	-13.11%	yes	yes
3	3	78	19	3	0	0	0	0	0.18	-2.46%	yes	yes
4	3	80	18	2	0	0	0	0	0.17	N/A	no	no
1	7	36	23	22	12	5	2	0	0.33	3.72%	yes	no
2	7	37	23	17	19	4	0	0	0.33	4.96%	yes	no
3	7	40	17	15	18	9	1	0	0.35	0.00%	yes	no
4	7	35	22	19	15	8	1	0	0.35	N/A	no	no
1	14	2	5	8	8	13	65	0	0.74	8.02%	yes	yes
2	14	2	3	5	9	8	72	1	0.77	4.44%	yes	yes
3	14	4	4	7	8	17	60	0	0.73	9.41%	yes	yes
4	14	0	0	6	5	13	72	4	0.80	N/A	no	no
1	17	0	0	2	3	6	89	0	0.83	2.51%	yes	yes
2	17	1	1	1	0	7	88	2	0.83	2.35%	yes	yes
3	17	1	2	0	0	9	88	0	0.83	3.18%	yes	yes
4	17	0	0	0	0	7	89	4	0.85	N/A	no	no
1	21	0	0	0	0	0	97	3	0.86	1.31%	yes	yes
2	21	0	0	0	0	0	97	3	10.86	1.31%	yes	yes
3	21	0	0	0	0	3	95	2	0.86	1.96%	yes	yes
4	21	0	0	0	0	1	87	12	0.87	N/A	no	no
1	28	0	0	0	0	0	85	15	0.88	2.84%	yes	yes
2	28	0	0	0	0	0	91	9	0.87	3.79%	yes	yes
3	28	0	0	0	0	0	81	19	0.88	2.21%	yes	yes
4	28	0	0	0	0	0	67	33	0.90	N/A	no	no
1	32	0	0	0	0	0	22	78	0.97	2.16%	yes	yes
2	32	0	0	0	0	0	16	84	0.98	1.30%	yes	yes
3	32	0	0	0	0	0	55	45	0.92	6.93%	yes	yes
4	32	0	0	0	0	0	7	93	0.99	N/A	no	no

Table 11 Cont.

Group	Days After Treatment	Grade 1	2	3	4	5	6	7	Index	Efficacy	T-test p<0.05	p<0.01
1	37	0	0	0	0	0	14	86	0.98	1.15%	yes	yes
2	37	0	0	0	0	0	7	93	0.00	0.14%	yes	yes
3	37	0	0	0	0	0	9	91	0.99	0.43%	yes	yes
4	37	0	0	0	0	0	6	94	0.99	N/A	no	no
1	42	0	0	0	0	0	12	88	0.98	1.01%	yes	yes
2	42	0	0	0	0	0	7	93	0.99	0.29%	yes	yes
3	42	0	0	0	0	0	8	92	0.99	0.43%	yes	yes
4	42	0	0	0	0	0	5	95	0.99	N/A	no	no
1	45	0	0	0	0	0	8	92	0.99	0.57%	no	no
2	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	45	0	0	0	0	0	4	96	0.99	N/A	no	no
1	50	0	0	0	0	0	7	93	0.99	0.43%	no	no
2	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	50	0	0	0	0	0	4	96	0.99	N/A	no	no

In previous trials tomatoes treated with Messenger® in the field and/or with a post harvest dip appeared to develop to red ripe more quickly, but decayed slower than the grower's standard. The data generated from this trial support these observations. By twenty-one days post harvest, 97% of the Messenger® treated tomatoes were full red ripe, compared to 87% of the grower's standard. Although it may be assumed that fruit which reach maturity more quickly will also start to break down more quickly, the results of the present Examples surprisingly demonstrate that these earlier-maturing tomatoes were actually 15% slower to decay than the grower's standard tomatoes. This phenomenon should be of great interest of several segments of the tomato market. The growers may be able to reduce ethylene gashouse timings, and the retail market should be able to significantly reduce inventory shrinkage.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:

treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

2. The method according to claim 1, wherein hypersensitive ~~response elicitor protein or polypeptide is in isolated form.~~

3. The method according to claim 2, wherein said treating is carried out prior to harvest of the fruit or vegetable.

4. The method according to claim 3, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

5. The method according to claim 4, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

6. The method according to claim 1, wherein said treating is carried out after harvest of the fruit or vegetable.

7. The method according to claim 6, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

8. The method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

9. The method according to claim 6, wherein said treating is carried out by immersing the fruit or vegetable in the hypersensitive response elicitor protein or polypeptide.

10. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia, Xanthomonas, Pseudomonas, Phytophthora*, and *Clavibacter*.

11. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

12. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

13. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

14. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

15. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

16. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

17. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

18. The method according to claim 1, wherein said treating inhibits desiccation in a fruit or vegetable.

19. The method according to claim 1, wherein said treating inhibits a postharvest disease in a fruit or vegetable.

20. The method according to claim 19, wherein the postharvest disease is caused by *Penicillium, Botrytis, Phytophthora,* or *Erwinia.*

21. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and

growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

22. The method according to claim 21, wherein a transgenic plant is provided.

23. The method according to claim 21, wherein a transgenic plant seed is provided.

24. The method according to claim 21, wherein the transgenic plant is a dicot or a monocot.

25. The method according to claim 21, further comprising:

applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to inhibit postharvest disease or desiccation.

26. The method according to claim 25, wherein said applying is carried out prior to harvest of the fruit or vegetable.

27. The method according to claim 25, wherein said applying is carried out after harvest of the fruit or vegetable.

28. The method according to claim 21, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

29. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

30. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

31. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

32. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

33. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

34. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

35. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

36. The method according to claim 21, wherein the postharvest disease is caused by *Penicillium*, *Botrytis*, *Phytophthora*, or *Erwinia*.

37. A DNA construct comprising:

a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide;

a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue; and

a 3' regulatory region operably coupled to the DNA molecule,

wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

38. An expression system comprising a vector into which is inserted a heterologous DNA construct according to claim 37.

39. A host cell comprising a heterologous DNA construct according to claim 37.

40. The host cell according to claim 39, wherein the host cell is a plant cell or a bacteria cell.

41. The host cell according to claim 40, wherein the bacteria cell is an *Agrobacterium* cell.

42. A transgenic plant comprising a heterologous DNA construct according to claim 37.

43. A method of enhancing the longevity of fruit or vegetable ripeness comprising:

treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness.

44. The method according to claim 43, wherein hypersensitive response elicitor protein or polypeptide is in isolated form.

45. The method according to claim 43, wherein said treating is carried out prior to harvest of the fruit or vegetable.

46. The method according to claim 43, wherein said treating is carried out after harvest of the fruit or vegetable.

47. A method of enhancing the longevity of fruit or vegetable ripeness comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and

growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant.

48. The method according to claim 47, further comprising:

applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness.

49. The method according to claim 48, wherein said applying is carried out prior to harvest of the fruit or vegetable.

50. The method according to claim 48, wherein said applying is carried out after harvest of the fruit or vegetable.

SEQUENCE LISTING

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Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
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Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
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Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
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Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln

260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
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325 330 335

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340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
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<213> Erwinia amylovora

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260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
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Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
355 360 365

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370 375 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385 390 395 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
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Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
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Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
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Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
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Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
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Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln

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Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
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Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
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Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
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Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
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Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
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245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr

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Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
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Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
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Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
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<213> Pseudomonas solanacearum

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Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
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Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
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Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
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Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
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Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
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Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
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Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
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Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
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Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
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(54) Title: HYPERSENSITIVE RESPONSE ELICITOR FROM *XANTHOMONAS CAMPESTRIS*

(57) Abstract

The present invention is directed to an isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or to controlling insects on plants. This can be achieved by applying the hypersensitive response elicitor in a non-infectious form to plants or plant seed under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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HYPERSENSITIVE RESPONSE ELICITOR FROM *XANTHOMONAS CAMPESTRIS*

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/103,124, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to a *Xanthomonas campestris* from hypersensitive response elicitor.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei,

Z.-M., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora*, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N$_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention identifies yet another hypersensitive response elicitor protein or polypeptide.

SUMMARY OF THE INVENTION

The present invention is directed to an isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.

The hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects. This involves applying the hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the hypersensitive response elicitor to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a hypersensitive response elicitor can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to an isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.

The hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling

insects. This involves applying the hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the hypersensitive response elicitor to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a hypersensitive response elicitor can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

The hypersensitive response elicitor polypeptide or protein derived from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

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Met Asp Gly Ile Gly Asn His Phe Ser Asn
 1               5                   10
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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 13-15 kDa.

Fragments of the above hypersensitive response elicitor polypeptides or proteins are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host *Xantamonas campestris*) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein hypersensitive response elicitor, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical

treatment. and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced

into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct

positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-

ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the hypersensitive response elicitor polypeptide or protein of the present invention, in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart disease resistance, impart stress resistance, enhance growth, and/or control insects. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to impart stress resistance, to enhance plant growth, and/or to effect insect control.

As an alternative to applying the hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding the hypersensitive response elicitor polypeptide or protein, which fragment elicits a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding the hypersensitive response elicitor polypeptide or protein of the present invention can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of the isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solancearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on

over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains the hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, the hypersensitive response elicitor of the present invention need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding the elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the

outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively,

transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which the hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including the hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

<u>Example 1</u> - Culture Growth

For the purpose of this study, *Xanthomonas campestris pelargonii (Xcp)* was grown on Luria agar plates. From these plates, colonies were transferred to inoculate *Xcp* seed cultures. The seed cultures were grown in 250ml baffled flasks containing 50ml of 50% Luria broth. The seed cultures were grown at approximately 27°C, while shaking at 250rpm, until an optical density (λ620) of 0.5 to 0.8 was reached.

To inoculate the minimal media, the seed cultures were centrifuged in sterile 500ml centrifuge bottles for 10 minutes at 4°C and 10,000 rpm. The

supernatant was discarded and the resulting cell pellet was resuspended in the already prepared minimal media, in a manner such that no Luria broth was introduced into the minimal media culture. A 1:10 ratio of seed culture to minimal media was consistently used for innoculating the minimal media. In other words, the cell pellet formed a 50ml seed culture which was used to inoculate 500ml of minimal media. The minimal media culture was grown in a 2.8L Fernbach flask containing 500ml of media at approximately 27°C which was agitated at 250 rpm until an optical density (λ620) of 1.7 to 2.0 was reached.

After the flask production of culture had been optimized, fermentation was transferred to 10L MicroFerm fermenter (New Brunswick Scientific, Edison, New Jersey, USA). For the 10L fermentation, the seed culture to minimal media ratio, as described above, was maintained. The fermentation was run at approximately 27°C with an initial pH of 6.0 and final pH of 5.8. The vessel was agitated at 400 rpm with 0.8 to 1.0 vessel volumes of air per minute.

A 1L 10x stock solution of the minimal media contained 39.2g of K_2HPO_4, 71.5g KH_2PO_4, 10.0g of $(NH_4)_2SO_4$, 3.5g of M_gCl_2, 1.0g of NaCl, and 34.23g of sucrose with a final pH of 6.0 to 6.2 was prepared after thoroughly mixing. The stock was sterile filtered and kept at 4°C.

Example 2 – Cell-Free Elicitor Preparation

The first step in the purification of the *Xcp* hypersensitive response elicitor was the development of a cell-free elicitor preparation ("CFEP"). The CFEP production involves four steps as described below.

1. Initial Centrifugation

In the initial centrifugation step, the minimal media cell culture was divided into 370ml aliquots and centrifuged in 500ml centrifuge bottles for 10 minutes, at 4°C, and at 8,000 rpm. The resulting cell pellet was resuspended at a 1:10 weight to volume ratio using lysis buffer. The lysis buffer consisted of 10mM NaCl and 20mM Tris-HCL at pH 8.0. Resuspension of the cell pellet was achieved

by vortexing individual centrifuge bottles. In the case of the 10L fermentation, a homogenizer was used.

2. Sonication

The resuspended pellet was then sonicated in 50ml aliquots at a setting of 5 for 3 minutes (with the horn tip, VirTris, VirSonic). While sonicating, the beaker containing the solution was immersed in an ice water bath. The resulting sonicate was kept on ice until all of the solution was sonicated. The setting used for sonication was the manufacturer's suggested maximum setting for the tip used.

3. Heat Treatment

The sonicate was placed on a preheated stir plate and brought to a rolling boil. The rolling boil was sustained for 5 minutes. After the 5 minute heat treatment, the solution was immediately placed in an ice water bath and cooled to approximately 10°C.

4. Final Centrifugation

Prior to final centrifuging, the cooled solution was brought back to its original volume with deionized H_2O, replacing the volume lost to evaporation during boiling. The solution was then centrifuged in 50ml centrifuge bottles for 30 minutes at 4°C and 15,000 rpm. The resulting supernatant from each bottle was combined and frozen in a –80°C freezer. A 1ml sample was also saved and used for testing hypersensitive response activity.

Example 3 - Protein Verification

To determine whether the hypersensitive response elicitor was indeed a protein, protease digestions were performed with CFEP. CFEP, prepared as previously described, was inoculated with protease K at a concentration of 2mg/ml. After a 1.5h incubation at 37°C, the protease inoculated CFEP along with the positive control (CFEP alone) and negative control (protease K at 2mg/ml in lysis buffer) were infiltrated into tobacco plants for hypersensitive response elicitor testing. The

positive control showed hypersensitive response necrosis, while the negative control showed no signs of testing. The protease K inoculated CFEP also showed no signs of hypersensitive response, indicating the *Xcp* hypersensitive response elicitor was sensitive to protease digestion and indeed a protein. This experiment was repeated several times with different batches of CFEP, each time with the same results.

Example 4 - Chromatographic Purification

The production of a hypersensitive response elicitor CFEP was the first step in the purification scheme for the *Xcp* hypersensitive response elicitor. Further purification of the elicitor consisted of four chromatographic steps. Anion exchange, cation exchange, various types of affinity chromatography, hydrophobic interaction, and reversed phase chromatography media were all analyzed for their utility in purifying the hypersensitive response elicitor. All chromatography experiments were conducted with the FPLC and FPLC detector (Pharmacia Biotech, Upsala, Sweden). The final purification scheme used consisted primarily of chromatography media that binded to the *Xcp* hypersensitive response elicitor based the hypersensitive response elicitor's hydrophobic characteristics.

1. Butyl Sepharose

The CFEP was first bound to a medium strength hydrophobic interaction chromatography medium. The CFEP was adjusted to 600mM NaCl and loaded on to a Butyl Sepharose 4 Fast Flow (Pharmacia Biotech, Uppsala, Sweden). The column was eluted with a 75-100% buffer B gradient. Buffer A contained 600mM NaCl, 20mM Tris-HC1 at pH 8, and buffer B contained 10mM Tris-HC1 at pH 8. At 85% B gradient, buffer B was exchanged for deionized H_2O.

2. Mono S

The fractions judged to have the highest concentration of the hypersensitive response elicitor (determined by making hypersensitive response dilution series with active fractions) from the Butyl Sepharose column were pooled together and loaded on to a strong cation exchanger, Mono S (Mono S 10/10 column,

Pharmacia Biotech). Prior to loading, the pooled fractions were adjusted to 20mM NaCl, 20mM Tris-HC1 at pH 5.5. Buffer A was 20mM NaCl, 20mM Tris-HC1 at ph 5.5. Buffer B contained 1M NaCl, 20mM Tris-HC1 at pH 5.5. The sample was loaded, followed by a 0% buffer B wash and then 100% buffer B wash. The hypersensitive response elicitor did not bind to the Mono S medium, but, at pH 5.5, many of the contaminates in the sample did. Thus, it served as a non-binding chromatography. Immediately following the collection of the flow through (the hypersensitive response elicitor fraction), the solution's pH was adjusted to 8.0.

3. Phenyl Sepharose (low)

The active fraction from the Mono S column was then loaded on to a Phenyl Sepharose 6 Fast Flow low substitution (a weak hydrophobic interaction medium, Pharmacia Biotech, Uppsala, Sweden). Buffer A contained 1M NaCl, 20mM Tris-HC1 at pH 8, and buffer B contained 10mM Tris-HC1 at pH 8.0. As mentioned previously, at 85% B, buffer B was changed to deionized H_2O. The hypersensitive response elicitor fraction was eluted as the gradient reached 100%B. The fractions which were then used in the next purification step contained the highest concentration of the hypersensitive response elicitor. Since the active fraction eluted in deionized H_2O, it was necessary to stabilize the fraction by adjusting them to 20mM NaCl and 20mM Tris-HC1 at pH 8.0.

4. Reversed Phase Chromatography

The final chromatographic step in the purification of the hypersensitive response elicitor utilized a ProRPC 5/5, reversed phase column (Pharmacia Biotech, Uppsala, Sweden). The sample was adjusted to 15% acetonitrile (HPLC grade) and 0.1% TFA (HPLC grade) and loaded at a flow rate of 0.7ml/min. After a 5ml wash, a 15-50% B gradient was run over 58ml at a flow rate of 0.7ml/min. 1ml fractions were collected for the entire volume of the gradient. The *Xcp* hypersensitive response elicitor eluted at approximately 25%B. Fractions containing the hypersensitive response elicitor were determined by visual identification on a silver stained PAGE-SDS gels. After visual identification, fractions that were relatively pure and contained high concentrations of the elicitor were pooled and dialyzed against the previously

mentioned lysis buffer to remove the acetonitrile and TFA. The dialyzed fraction was then concentrated approximately 100 fold using a Centricon 3,000 MWCO (Amicon, Beverly, MA).

Example 5 - Electroelution of the *Xcp* Hypersensitive Response Elicitor

The final concentrate was then run on a hand poured 18% acrylimide PAGE-SDS mini gel (gel was poured in an empty Novex gel cassette and run with a Novex X-Cell apparatus, San Diego, CA). The gel was stained and destained with normal Coomassie Blue staining techniques. Because of extreme overloading of the gel, it was possible to see and cut out the band that correlated with the hypersensitive response elicitor. The cut out gel was then loaded into an Elutrap (Schleicher & Schuell, Keene, New Hampshire). The protein present on the cut out was eluted off the gel, and into a collection chamber. A non-SDS tank buffer was used in the Elutrap to produce a sample relatively free of SDS. A portion of the resulting fraction was then run on a 16% acrylimide PAGE-SDS mini gel (Novex gel and apparatus). The gel was silver stained in order to determine the sample's purity. In addition, a portion of the eluted fraction was used to make hypersensitive response elicitor dilutions to determine the relative concentration of hypersensitive response elicitor present in the final sample.

Example 6 - Visualization of the Hypersensitive Response Elicitor

Perhaps the most significant and troublesome characteristic encountered during the isolation of the *Xcp* hypersensitive response elicitor was its abnormal staining characteristics. PAGE-SDS gels that had been stained and destained using normal Coomassie Blue techniques showed the hypersensitive response elicitor band only when the gel was extremely overloaded. Even under overloaded conditions, the elicitor stained very faintly and to some extent temporarily. Complete destaining of the gel usually resulted in fading of the hypersensitive response elicitor band. Only with silver staining techniques was it possible to visualize the elicitor at a seemingly lower concentration. The band, depending of the concentration of the hypersensitive response elicitor present in the sample, either

appeared as a negatively stained band with relatively distinct borders (at higher concentration) or as a slightly off-colored band, almost resembling a shadow with non-distinct, fuzzy borders. Silver stained PAGE-SDS gels were used to visualize the hypersensitive response elicitor in all cases, except when the gel was to be used for electroelution with the Elutrap or protein transfer on to a PVDF membrane.

As in the case of Coomassie Blue staining of PAGE-SDS gels, Coomassie Blue staining of PVDF membranes (used to immobilize the protein for N-terminal sequencing) resulted in very faint staining if intensely overloaded. Normal loading volumes resulted in a seemingly clean membrane with no apparent protein bands.

Example 7 - N-terminal Sequence of the *Xcp* Hypersensitive Response Elicitor

After purification of the elicitor had been achieved it was then possible to learn the N-terminal sequence of the protein. The purified *Xcp* hypersensitive response elicitor protein was run on a SDS-PAGE. From this gel, the protein was transferred to a PVDF membrane via a Trans-Blot (Bio-Rad, Hercules, CA). The membrane containing the protein was subjected to an automated Edmans Degradation process followed by high-pressure liquid chromatography for detection and identification of the individual amino acids. The first ten amino acids of the *Xcp* hypersensitive response elicitor were determined to be:

```
Met Asp Gly Ile Gly Asn His Phe Ser Asn
 1               5                   10
```

(SEQ. ID. No. 1).

Example 8 - Elicitor-Induced Disease Resistance in Tobacco and Tomato

The hypersensitive response elicitor of *Xanthomonas campestris pv. pelargonii* was sprayed on tobacco plants at a concentration of approximately 5 ppm. Three days after the treatment, the plants were inoculated with tobacco mosaic virus (TMV) at a concentration of ca. 2 ppm. Four days after inoculation, the elicitor treated plants showed more than a 65% reduction in the number of lesions compared

to untreated control plants. In addition to resistance to TMV, the hypersensitive response elicitor also induced resistance to bacterial wilt of tomato.

Example 9 - The Hypersensitive Response Elicitor-Induced Growth Enhancement of Tomato

To demonstrate that the hypersensitive response elicitor of *Xanthomonas campestris pv. pelargonii* can enhance plant growth, tomato seeds were soaked in an elicitor solution of ca. 5 ppm for more than 4 hours. The seeds soaked in the same solution but without the elicitor protein were used as untreated control. Both the treated and untreated seeds were planted in eight inch pots with artificial soil, 20 seeds per pot. Twenty days after planting, the size of the tomato seedlings were measured. The plants derived from the elicitor treated seeds were 10% fuller than those generated from the untreated seeds.

A proteinaceous hypersensitive response elicitor has been purified to near homogeneity from the plant pathogen *Xanthomonas campestris pelargonii*. The protein has a molecular weight of approximately 14kDa and is heat tolerant. The hypersensitive response elicitor exhibits unusual staining characteristics when stained with normal Coomassie Blue and silver staining techniques. The *Xanthomonas campestris pelargonii* hypersensitive response elicitor is a member of the hypersensitive response elicitor protein family due to its shared characteristics with other proteins of this family. These characteristics include biochemical characteristics, the ability to elicit a hypersensitive response, and, from preliminary experiments, the ability to induce disease resistance and plant growth enhancement.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

WHAT IS CLAIMED:

1. An isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.

2. An isolated hypersensitive response elicitor protein or polypeptide according to claim 1, wherein the protein or polypeptide has a molecular weight of 13-15 kDa.

3. ~~An isolated hypersensitive response elicitor protein or~~ polypeptide according to claim 1, wherein the protein or polypeptide has an amino acid sequence of SEQ. ID. No. 1.

4. A method of imparting disease resistance to plants comprising:

applying a hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

5. A method according to claim 4, wherein plants are treated during said applying.

6. A method according to claim 4, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

7. A method of enhancing plant growth comprising:

applying a hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.

8. A method according to claim 7, wherein plants are treated during said applying.

9. A method according to claim 7, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

10. A method of insect control for plants comprising:

applying hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to control insects.

11. A method according to claim 10, wherein plants are treated during said applying.

12. A method according to claim 10, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

SEQUENCE LISTING

<110> Eden Bioscience Corporation

<120> HYPERSENSITIVE RESPONSE ELICITOR FROM XANTHOMONAS CAMPESTRIS

<130> 21829/52

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 99/23265

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SWANSON, S. ET AL.: "Isolation and characterization of an HR elicitor from Xanthomonas campestris." PHYTOPATHOLOGY, vol. 88, no. 9 SUPPL., September 1998 (1998-09), page S87 XP000866309	1-3
Y	the whole document ---	4-12
X	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11)	1
Y	page 12, line 16 -page 13, line 25 page 25, line 39 -page 26, line 16 abstract ---	4-6
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2000	20/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mata Vicente, T.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23265

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) page 9, line 3 -page 10, line 7	1
Y	page 22, line 9 - line 37 abstract	7-9

X	WO 98 37752 A (CORNELL RES FOUNDATION INC) 3 September 1998 (1998-09-03)	1
Y	page 3, line 27 -page 4, line 29 page 16, line 9 - line 37	10-12

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/23265

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9824297 A	11-06-1998	AU 5693598 A	29-06-1998
		EP 0957672 A	24-11-1999
WO 9832844 A	30-07-1998	AU 6043198 A	18-08-1998
WO 9837752 A	03-09-1998	AU 6666498 A	18-09-1998

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(54) Title: USE OF HYPERSENSITIVE RESPONSE ELICITOR FROM GRAM POSITIVE BACTERIA

(57) Abstract

The present invention is directed to the use of a protein or polypeptide from Gram positive bacteria, such as *Clavibacter michiganensis* subsp. *sepedonicus*, which elicits a hypersensitive response in plants. This protein or polypeptide can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects on plants. This can be achieved by applying the hypersensitive response elicitor protein or polypeptide in a non–infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

USE OF HYPERSENSITIVE RESPONSE ELICITOR FROM GRAM POSITIVE BACTERIA

FIELD OF THE INVENTION

The present invention relates to the use of the hypersensitive response elicitor from Gram positive bacteria, such as *Clavibacter michiganensis* subsp. *sepedonicus,* for disease resistance, growth enhancement, and insect control.

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/057,464, filed September 3, 1997.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response ("HR") is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower

levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al., "Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, hrp genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas

Syringae pv. Syringae Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N$_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention is directed to the use of a hypersensitive response elicitor protein or polypeptide from Gram positive bacteria.

SUMMARY OF THE INVENTION

The hypersensitive response eliciting protein or polypeptide from Gram positive bacteria, such as *Clavibacter*, particularly *Clavibacter michiganensis* subsp. *sepedonicus*, can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects. This involves applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the gram positive hypersensitive response elicitor protein or polypeptide to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding the hypersensitive response gram positive elicitor protein or polypeptide and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding the gram positive hypersensitive response elicitor protein or polypeptide can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the hypersensitive response elicitor protein or polypeptide from Gram positive bacteria, such as a *Clavibacter*, particularly *Clavibacter michiganensis* subsp. *sepedonicus*, for disease resistance, growth enhancement, and insect control in plants. This hypersensitive response

elicitor protein or polypeptide is described in and can be isolated according to Nissinen, et al., "*Clavibacter michiganensis* subsp. *sepedonicus* Elicits a Hypersensitive Response in Tobacco and Secretes Hypersensitive Response-Inducing Protein(s)," Phytopathology, pp. 678-84 (1997), which is hereby incorporated by reference.

The gene encoding this hypersensitive response elicitor protein or polypeptide can be obtained by procedures well known in the art. The protein can be purified through conventional techniques such as chromatography or electrophoresis. The amino terminal sequence of the protein is determined and used to design degenerate oligonucleotides which are labelled and used as probes to screen a clone library. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), which is hereby incorporated by reference. Plasmid DNA is isolated and sequenced from clones which hybridize to the amino terminal probe. The sequence of the DNA molecule can be determined using either chemical (Maxam et al., Proc. Natl. Acad. Sci. USA, 74:560 (1977), which is hereby incorporated by reference) or enzymatic (Sanger, et al., Proc. Natl. Acad. Sci. USA, 74:5463 (1977), which is hereby incorporated by reference) methods.

Fragments of the above hypersensitive response elicitor polypeptide or protein are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the elicitor protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, differential pressure, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant

DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria

transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its

bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be isolated from its bacterial source.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seed cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are:

Arabidopsis thaliana, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solancearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved

crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, and tomato pinworm. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include topical applications, such as high or low pressure spraying, as well as injection and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by topical procedures, such as low or high pressure spraying, coating, or immersion, as well as by injection. Other suitable application

procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive

response elicitor polypeptide or protein are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes

allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing

transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart

disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

Although the invention has been described in detail for the purpose of illustration, it is understood that such details are solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit of the scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of imparting disease resistance to plants comprising:

applying a hypersensitive response elicitor protein or polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or plant seed under conditions where the protein or polypeptide contacts the cells of the plants or the plant seeds and imparts disease resistance.

2. A method according to claim 1, wherein plants are treated during said applying.

3. A method according to claim 1, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

4. A method according to claim 1, wherein the Gram positive bacterium is a *Clavibacter*.

5. A method according to claim 4, wherein the *Clavibacter* is *Clavibacter michiganensis* subsp. *sepedonicus*.

6. A method of enhancing plant growth comprising:

applying a hypersensitive response elicitor protein or polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or plant seed under conditions where the protein or polypeptide contacts the cells of the plants or the plant seeds and enhances plant growth.

7. A method according to claim 6, wherein plants are treated during said applying.

8. A method according to claim 6, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

9. A method according to claim 6, wherein the Gram postiive bacterium is a *Clavibacter*.

10. A method according to claim 9 wherein the *Clavibacter* is *Clavibacter michiganensis* subsp. *sepedonicus*.

11. A method of insect control for plants comprising:

applying a hypersensitive response elicitor protein or polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or plant seed under conditions where the protein or polypeptide contacts the cells of the plants or the plant seeds and controls insects.

12. A method according to claim 11, wherein plants are treated during said applying.

13. A method according to claim 11, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

14. A method according to claim 11, wherein the Gram positive bacterium is a *Clavibacter*.

15. A method according to claim 14, wherein the *Clavibacter* is *Clavibacter michiganensis* subsp. *sepedonicus*.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/17252

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 09114 A (CROP GENETICS INTERNATIONAL) 1 December 1988 see page 4, line 1 - line 111 see page 6, line 1 - line 12 see page 6, line 28 - page 8, line 5	1-15
X	WO 90 13224 A (CROP GENETICS INTERNATIONAL) 15 November 1990 see page 3, line 26 - page 4, line 14 see page 6, line 25 - line 36 see examples 1-11	1-15
X	WO 91 10363 A (CROP GENETICS INTERNATIONAL) 25 July 1991 see the whole document	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 1998	21/12/1998

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	CHEMICAL ABSTRACTS, vol. 120, no. 15, 11 April 1994 Columbus, Ohio, US; abstract no. 184202, LAMPEL, JAY S. ET AL: "Integrative cloning, expression, and stability of the cryIA(c) gene from Bacillus thuringiensis subsp. kurstaki in a recombinant strain of Clavibacter xyli subsp. cynodontis" XP002086718 see abstract & APPL. ENVIRON. MICROBIOL. (1994), 60(2), 501-8 CODEN: AEMIDF;ISSN: 0099-2240,	1-15
X	CHEMICAL ABSTRACTS, vol. 127, no. 4, 28 July 1997 Columbus, Ohio, US; abstract no. 47639, METZLER, MARY C. ET AL: "The status of molecular biological research on the plant pathogenic genus Clavibacter" XP002086719 see abstract & FEMS MICROBIOL. LETT. (1997), 150(1), 1-8 CODEN: FMLED7;ISSN: 0378-1097,	1-15
X	BIOLOGICAL ABSTRACTS, vol. 95, Philadelphia, PA, US; abstract no. 452043, TOMASINO S F ET AL: "Field performance of Clavibacter xyli subsp. cynodontis expressing the insecticidal protein gene cryIA(c) of Bacillus thuringiensis against European corn borer in field corn." XP002086716 see abstract & BIOLOGICAL CONTROL 5 (3). 1995. 442-448. ISSN: 1049-9644,	1-15

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICAL ABSTRACTS, vol. 95, Philadelphia, PA, US; abstract no. 350265, NISSINEN R ET AL: "Clavibacter michiganensis subsp. sepedonicus elicits a hypersensitive response in tobacco and secretes hypersensitive response-inducing protein (s)." XP002086717 see abstract & PHYTOPATHOLOGY 87 (7). 1997. 678-684. ISSN: 0031-949X, cited in the application -----	1-15

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INTERNATIONAL SEARCH REPORT

...ormation on patent family members

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